# B I OS A F E T Y  M A N U A L

Centers for Disease Control and Prevention
National Center for Infectious Diseases
Division of Vector-borne Infectious Diseases

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Fort Collins, Colorado 80521
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# FOREWORD

Safety in the workplace at the Division of Vector-Borne Infectious Diseases is a priority that we all take seriously. As will be evident in this manual, safety in this facility involves many different considerations. Employees must be aware of issues in biosafety, chemical safety, radiation safety, and building safety. This manual attempts to present, in a clear manner, safety procedures that must be followed when working in this Division; it was produced with considerable effort by the Biosafety Committee, the Branch Chiefs, and the Division Director. It is intended to be a "hands-on" working document to be used as a reference and a teaching tool for new employees and visitors. Your comments about the content and organization of this manual are encouraged.

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ACKNOWLEDGMENTS

Major portions of this manual have been adapted from existing material on biosafety standards. Chief among these is Biosafety in Microbiological and Biomedical Laboratories, published jointly by the Centers for Disease Control (CDC) and the National Institutes of Health. Additional procedures have been contributed by the Office of Health and Safety, Centers for Disease Control and Prevention, Atlanta, Georgia, and radiation safety guidelines produced by Colorado State University. Safety standards for particular procedures in this Division were the efforts of Branch Chiefs, Section Chiefs, laboratorians, and members of the Biosafety Committee.

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# GENERAL BIOSAFETY PRINCIPLES

## BUILDING SECURITY

Building security measures in this facility are designed to 1) prevent exposure of unvaccinated persons to infectious agents and 2) prevent unauthorized entry.

All external doors to this building are to remain locked 24 hours a day. Personnel requiring access will be issued a key. This key is an accountable item and may not be loaned nor duplicated. Lost or stolen keys must be reported immediately to the Program Analyst.

Unauthorized and unvaccinated visitors are not allowed in restricted laboratory areas. Children under age 12 are not allowed in the building at any time. Pets are not allowed in the building at any time. Immunized visitors in restricted areas should be escorted at all times. Unvaccinated visitors are only allowed in the second-floor entrance area, on the third floor, or in other unrestricted areas such as offices on the east side of the second floor.

IDENTIFICATION BADGES: All persons in this building should wear an identification badge at all times. Permanent employees, part-time employees, and visiting scientists will be issued a badge that includes an individual photograph. Short-term visitors and salesmen/tradesman must sign in at the reception desk in the second-floor reception area, and will be issued temporary passes, which should be returned when they sign out upon their departure. Arrangements to secure these passes should be made through the Program Analyst.

## FIRE SAFETY

The CSU Fire Emergency Number is 9-491-7111.

This building is equipped with automatic smoke and heat-activated alarms. When an alarm occurs, the CSU emergency center is automatically alerted and calls the lab to verify an alarm received during normal duty hours. This arrangement allows verification that the alarm has been received at CSU. If you hear the alarm outside normal duty hours, call the above number to verify CSU's reception of the alarm. This is helpful because all incoming calls during off-duty hours are diverted to a single third-floor telephone and the confirming call from CSU may not be answered. The Program Analyst and Division Director should be notified at home of fire occurring during off-duty hours.

Two types of alarms are used in this facility --- a continuous, loud, audible alarm and flashing visual alarms for the hearing impaired. Before exiting, be sure that no one is left in a photography darkroom, a walk-in freezer, or at a biosafety cabinet. **WALK** quickly to the

nearest exit. Do not use the elevator.

There are fire control stations on each floor containing fire extinguishers and manual switches for the alarm system. If you discover a fire, no matter what size the fire, activate the manual switch if the automatic alarms have not sounded. Do not attempt to extinguish a fire yourself unless it is a very small one. Remember that many laboratories contain flammable reagents and what may begin as a small fire can escalate to deadly levels in a matter of seconds. Memorize the locations of fire extinguishers and fire control stations in your work areas.

Floor plans with EXITS marked are mounted on the walls at numerous locations on each floor of the main building. Take time to familiarize yourself with the locations of a primary and alternative escape routes from each floor. All doors that can be used as EMERGENCY EXITS (including some doors not in routine traffic use) are marked by illuminated and nonilluminated EXIT signs.

## ESCAPE FROM WALK-IN FREEZERS, REFRIGERATORS, AND INCUBATORS

Walk-in refrigerators, freezers, and incubators are located in various laboratories on the second floor. These walk-ins do not have normal door knobs, and the opener on the inside is different from the outer one. The inner door opener is a metal rod that protrudes out and ends in a metal disk. Pushing this disk toward the door will open the door from the inside. Familiarize yourself with the location and operation of this disk before entering a walk-in.

## MATERIAL SAFETY DATA SHEETS (MSDS)

The chemical industry in the United States, by law, must provide safety information along with all chemicals sold. These sheets (MSDS) contain valuable information on acute toxicity, teratogenicity, carcinogenicity and flammability. Read the MSDS for each chemical used and take the required safety precautions. A master MSDS file is kept by the Biosafety Officer. Contents of this file are stored alphabetically by chemical name. You may build your personal MSDS file by copying the master file or by retaining the MSDS that accompany each chemical order.

## HIGH-SECURITY LABORATORY

Room 208 contains the High-Security Laboratory for this facility. It is designed as a Biosafety Level 3 laboratory and has the following features: 1) limited access through key-locked, double-door, outer rooms containing lockers and showers; 2) isolated air handling system; 3) dedicated pass-through autoclave and pass-through, locking, double doors.

The High-Security Laboratory serves all diagnostic and research components of the Division. To fairly allocate this resource, its use must be scheduled with the Utilization Coordinator whose

name appears on the outer door. This should be done as far in advance of actual need as possible. After scheduling a use time, complete and forward to the Biosafety Officer a "Notice of Intent to Work with a Hazardous Biological Agent or Toxic Material" form (CDC 0.838, Rev. 2-85). Copies of this form are available from the Biosafety Officer.

**Transfer of Biohazardous Agents into the High-Security Laboratory.**

Moving biohazardous materials into the High-Security Laboratory from other parts of the building entails a risk of breakage and spills. The following guidelines should be strictly adhered to when moving infectious agents.

1. The primary infectious agent container must be placed in an unbreakable second container. Unless the primary container is too large, it should be placed in a screw-top or snap-top secondary container.

2. Large primary containers should be placed in a plastic tub double-wrapped with autoclave bags, both of which are to be sealed tightly with ties.

3. All cell culture flasks and all secondary containers of size 250 ml or greater should be transported on a cart.

4. No infectious agents should be transported on stairs. Use elevators.

5. Infectious materials should be introduced into the laboratory via the double pass-through doors. They should **NOT** be brought in through the laboratory entrance air-lock double doors.

**Entering the High-Security Laboratory.**

The High-Security Laboratory should be entered only by persons actually working there. Persons working in the laboratory may be reached at phone extension 6451; however, they should not be disturbed unless absolutely necessary.

Entrances to the airlock doors are located in the rear of the men’s and women’s lavatories across from Rooms 201 and 206, respectively. The airlocks contain

showers, shampoo, soap, towels, and clothing lockers. Scrub suits and shoes or shoe covers are located in a cabinet in the hallway.

Shoes, socks, stockings, and all outer and under clothing must always be removed and replaced with scrub suits, shoes or shoe covers, and undergarments provided. Under certain circumstances, some of these clothing requirements may be waived, provided they are approved in advance by the Biosafety Officer. Before beginning work in the laboratory, the laboratorian must have the following safety items: laboratory coat, surgical gloves, scrub hat, safety glasses, surgical mask, and, when appropriate, a respirator. When the person is ready to exit the High-Security Laboratory, all protective gear and all clothing worn must be bagged or otherwise prepared for autoclaving. A thorough shower must be taken and hair must be shampooed before dressing in personal clothing. All glassware and other items used in the laboratory must be sterilized in an autoclave before removal.

Spills or any other types of accidents that occur in the High-Security Laboratory should be reported immediately to the Biosafety Officer. Refer to the section on spills (page 78). Fire extinguishers are located inside the laboratory for small fire emergencies. Refer to the section on fire safety (page 1).

When not in use as a High-Security Laboratory, Room 208 may be used as a Security Level 2 laboratory. Under these circumstances, entrance can be made through the hallway door as with other laboratories. Under certain circumstances, both Biosafety Level 2 and Biosafety Level 3 agents may be worked on in Room 208 at the same time. When this happens, the laboratory and all agents will be treated as Class III, and all procedures outlined above for the High-Security Laboratory must be followed.

# GENERAL LABORATORY BIOSAFETY

## LABORATORY BIOSAFETY LEVEL CRITERIA

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in the discussions which follow. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

**Biosafety Level 2** is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment. The following standard and special practices for safety equipment and facilities apply to agents assigned to Biosafety Level 2.

Standard Microbiological Practices

1. Access to the laboratory is limited or restricted by the laboratory supervisor when work with infectious agents is in progress.

2. Work surfaces are decontaminated at least once a day and after any spill of viable material.

3. All infectious liquid or solid wastes are decontaminated before disposal.

4. Mechanical pipetting devices are used; pipetting by mouth is prohibited.

5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.

6. Persons must wash their hands after handling infectious materials and animals and when they leave the laboratory.

7. All procedures are performed carefully to minimize the creation of aerosols.

Special Practices

1. Contaminated materials that are to be decontaminated at a site away from the

laboratory are placed in a durable leakproof container, which is closed before being removed from the laboratory.

2. The laboratory supervisor limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

3. The laboratory supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

4. When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory supervisor or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

5. An insect and rodent control program is in effect.

6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative office), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

7. Animals not involved in the work being performed are not permitted in the laboratory.

8. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

9. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles

should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated by autoclaving before discard or reuse.

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory supervisor and to the Division's Biosafety Officer. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.

12. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

Containment Equipment

Biological safety cabinets (Class II) (see Appendix A) or other appropriate personal protective or physical containment devices are used in the following circumstances:

1. Procedures with a high potential for creating infectious aerosols are being conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious material whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

2. High concentrations or large volumes of infectious agents are often used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Laboratory Facilities

1. The laboratory is designed so that it can be easily cleaned.

2. Benchtops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

3. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

4. Each laboratory contains a sink for handwashing.

5. An autoclave for decontaminating infectious laboratory wastes is available.

**Biosafety Level 3** is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facilities apply to agents assigned to Biosafety Level 3.

Standard Microbiological Practices

Refer to "Entering the High-Security Laboratory" on page 3.

1. Work surfaces are decontaminated at least once a day and after any spill of viable material.

2. All infectious liquid and solid wastes are decontaminated before removal from the High Security Laboratory and disposal.

3. Mechanical pipetting devices are used; mouth pipetting is prohibited.

4. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

5. Persons wash their hands after handling infectious materials and animals, and must shower and change clothes before they leave the laboratory.

6. All procedures are performed carefully to minimize the creation of aerosols.

Special Practices

1. Laboratory doors are kept locked when experiments are in progress.

2. Contaminated materials are decontaminated before leaving the laboratory and are placed in a durable leakproof container which is closed before being autoclaved and removed from the laboratory.

3. The laboratory supervisor controls access to the laboratory and restricts access to persons whose presence is required. Persons for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

4. The laboratory supervisor establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures enter the laboratory or animal rooms.

5. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory supervisor or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

6. All activities involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

8. An insect and rodent control program is in effect.

9. Laboratory clothing (scrub suits, coveralls) is changed into and worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.

10. Special care is taken to avoid skin contamination with infectious materials; gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.

11. Molded surgical masks or respirators are worn in rooms containing infected animals.

12. Animals and plants not related to the work being conducted are not permitted in the laboratory.

13. All wastes from laboratories and animal rooms are appropriately decontaminated before removal and disposal.

14. Vacuum lines are protected with high-efficiency particulate air (HEPA) filters and liquid disinfectant traps.

15. Hypodermic needles and syringes are used only for parenteral diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

16. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory supervisor and to the Division Biosafety Officer. Appropriate medical evaluation, surveillance, and treatment are provided, and written records are maintained.

17. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.

Containment Equipment

Biological safety cabinets Class II or III (see Appendix A) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs; and necropsy of infected animals.

Laboratory Facilities

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high-containment laboratory from access corridors or other laboratories or activities may also be provided by a clothes-changing room with double doors (showers are included), airlock, or other access facility that requires passage through two sets of doors before entering the laboratory.

2. The interior surfaces of walls, floors, and ceilings are water-resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

5. Each laboratory contains a sink for handwashing. The sink is foot, elbow, or automatically operated, and is located near the laboratory exit door.

6. Windows in the laboratory are closed and sealed.

7. Access doors to the laboratory or containment module are self-closing.

8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.

9. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room can be discharged to the outside without being filtered or otherwise treated.

10. The HEPA-filtered exhaust air from Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class II biological safety cabinets is to be discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

## VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA

If experimental animals are used, institutional management must provide facilities and staff and establish practices that reasonably ensure appropriate levels of environmental quality, safety, and care. Laboratory animal facilities are extensions of the laboratory and in some situations are integral to and inseparable from the laboratory. As a general principle, the Biosafety Level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations and that appropriate species have been selected for animal experiments (e.g., Guide for the Care and Use of Laboratory Animals, HEW Publication No. (1) 78-23, Rev. 1978, and Laboratory Animal Welfare Regulations - 9 CFR, Subchapter A, Parts 1, 2, and 3). Ideally, facilities for laboratory animals used for studies of infectious or noninfectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities that provide patient care. Animal facilities should be designed and constructed to facilitate cleaning and housekeeping. A "clean hall/dirty hall" layout is very useful in reducing cross-contamination. Floor drains should be installed in animal facilities only on the basis of clearly defined needs. If floor drains are installed, the drain trap should always contain water.

These recommendations describe four combinations of practices, safety equipment, and facilities for experiments on animals infected with agents that are known or believed to produce infections in humans. These four combinations provide increasing levels of protection to personnel and to the environment and are recommended as minimal standards for activities involving infected laboratory animals. These four combinations, designated Animal Biosafety Levels 1-3, describe animal facilities and practices applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-3.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in standards written for commonly used laboratory animals. "Laboratory Safety for Arboviruses and Certain Other Viruses of Vertebrates", prepared by the Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses, serves as a useful reference in the design and operation of facilities using arthropods.

**Animal Biosafety Level 1**

Standard Practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.

2. Work surfaces are decontaminated after use or after any spill of viable materials.

3. Eating, drinking, smoking, and storing food for human use are not permitted in animal rooms.

4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.

5. All procedures are carefully performed to minimize the creation of aerosols.

6. An insect and rodent control program is in effect.

Special Practices

1. Bedding materials from animal cages are removed in a manner that minimizes the creation of aerosols and disposed of in compliance with applicable institutional or local requirements.

2. Cages are washed manually or in a cage washer. The temperature of final rinse water in a mechanical washer should be 180°F.

3. Wearing laboratory coats, gowns, or uniforms in the animal room is recommended. It is further recommended that laboratory coats worn in the animal room not be worn in other areas.

Containment Equipment

Special containment equipment is not required for animals infected with agents assigned to Biosafety Level 1.

Animal Facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.

2. A sink for washing hands is available in the animal facility.

3. It is recommended, but not required, that the direction of airflow in the animal facility be inward and that exhaust air be discharged to the outside without being recirculated to other rooms.

**Animal Biosafety Level 2**

Standard Practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when infected animals are present.

2. Work surfaces are decontaminated after use or spills of viable materials.

3. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.

4. Personnel wash their hands after handling cultures and animals.

5. All procedures are carefully performed to minimize the creation of aerosols.

6. An insect and rodent control program is in effect.

Special Practices

1. Cages are decontaminated, preferably by autoclaving, before they are cleaned and washed.

2. Surgical-type masks are to be worn by all personnel entering animal rooms housing nonhuman primates.

3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.

4. The laboratory or animal facility supervisor limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.

5. The laboratory or animal facility supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., for immunization) may enter the animal room.

6. When the infectious agent(s) being worked with in the animal room requires special entry provisions (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the animal facility and indicates the special requirement(s) for entering the animal room.

7. Special care is taken to avoid skin contamination with infectious materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

8. All wastes from the animal room are appropriately decontaminated -- preferably by autoclaving -- before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.

9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

10. If floor drains are provided, the drain traps must always be filled with water or a suitable disinfectant.

11. When appropriate, considering the agents handled, baseline serum samples from animal care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or

the function of the facility.

Containment Equipment

1. Biological safety cabinets, other physical containment devices, and/or personal protective devices (e.g., respirators, face shields) are used whenever procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulations of high concentrations or large volumes of infectious materials.

Animal Facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.

2. A sink for washing hands is available in the room where infected animals are housed.

3. It is recommended, but not required, that the direction of airflow in the animal facility be inward and that exhaust air be discharged to the outside without being recirculated to other rooms.

4. An autoclave that can be used for decontaminating infectious laboratory waste is available in the building with the animal facility.

**Animal Biosafety Level 3**

Standard Practices

1. Doors to animal rooms open inward, are self-closing, and are kept locked when work with infected animals is in progress.

2. Work surfaces are decontaminated after use or spills of viable materials.

3. Eating, drinking, smoking, and storing of food for human use are not permitted in the animal room.

4. Personnel wash their hands after handling cultures and animals and before leaving the laboratory.

5. All procedures are carefully performed to minimize the creation of aerosols.

6. An insect and rodent control program is in effect.

Special Practices

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.

2. Surgical-type masks or other respiratory protection devices (e.g., respirators) are worn by personnel entering rooms housing animals infected with agents assigned to Biosafety Level 3.

3. Scrub suits provided must be changed into and worn by personnel entering the animal room. Protective gowns and scrub suits must remain in the animal room and must be decontaminated before being laundered.

4. The laboratory supervisor or other responsible person restricts access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.

5. The laboratory supervisor or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., for immunization) may enter the animal room.

6. Biohazard warning signs, incorporating the universal biohazard warning symbol, are posted on access doors to animal rooms containing animals infected with agents assigned to Biosafety Level 3. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal room supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for immunizations or respirators).

7. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before being disposed of or reused.

8. All wastes from the animal room are autoclaved before disposal.

   All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leakproof covered containers.

9. Hypodermic needles and syringes are used only for gavage or for parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable needle syringe units (i.e., the needle is integral to the syringe) are used. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse. Whenever possible, cannulas should be used instead of sharp needles (e.g., gavage).

10. Vacuum lines are protected with HEPA filters and liquid disinfectant traps.

11. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.

Containment Equipment

1. Personal protective clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of infectious materials or infected animals.

2. The risk of infectious materials or infected animals or their bedding can be reduced if animals are housed in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar flow cabinets), solid wall and bottom cages covered by filter bonnets, or other equivalent primary containment systems.

## RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS AND INFECTED ANIMALS

Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors. Some of the most important are: the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; and the availability of effective vaccines or therapeutic measures.

Agent summary statements in this section provide guidance for the selection of appropriate biosafety levels. Specific information on laboratory hazards associated with a particular agent and recommendations regarding practical safeguards that can significantly reduce the risk of laboratory-associated diseases are included. Agent summary statements are presented for agents which meet one or more of the following criteria: the agent is a proven hazard to laboratory personnel working with infectious materials (e.g., hepatitis B virus, tubercle bacilli); the potential for laboratory-associated infections (e.g., exotic arboviruses); or, the consequences of infection are grave (e.g., Creutzfeldt-Jakob disease, botulism).

Recommendations for the use of vaccines and toxoids are included in agent summary statements when such products are available -- either as licensed or Investigational New Drug (IND) products. When applicable, recommendations for the use of these products are based on current recommendations of the Public Health Service Advisory Committee on Immunization Practice and are specifically targeted to at-risk laboratory personnel and others who must work in or enter laboratory areas. These specific recommendations should in no way preclude the routine use of such products as diphtheria-tetanus toxoids, poliovirus vaccine, influenza vaccine, and others because of the potential risk of community exposures irrespective of any laboratory risks. Appropriate precautions should be taken in the administration of live attenuated virus vaccine in individuals with altered immunocompetence.

Risk assessments and biosafety levels recommended in the agent summary statements presuppose a population of immunocompetent individuals. Those with altered immunocompetence may be at increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital, or induced by a number of neoplastic diseases, by therapy, or by radiation. The risk of becoming infected or the consequences of infection may also be influenced by such factors as age, sex, race, pregnancy, surgery (e.g., splenectomy, gastrectomy), predisposing diseases (e.g., diabetes, lupus erythematosus), or altered physiological function. These and other variables must be considered in individualizing the generic risk assessments of the agent summary statements for specific activities. The basic biosafety level assigned to an agent is based on the activities typically associated with growth and manipulations of quantities and concentrations of infectious agents required to accomplish identification or typing. If activities with clinical materials pose a lower risk to personnel than those activities associated with manipulation of cultures, a lower biosafety level is recommended. On the other hand, if the activities involve large volumes or highly concentrated preparations ("production quantities") or manipulations that are likely to produce aerosols or that are otherwise intrinsically hazardous, additional personnel precautions and increased levels of primary and secondary containment may be indicated. "Production quantities" refers to large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities. Propagation and concentration of infectious agents which occurs in large-scale fermentations, antigen and vaccine production, and a variety of other commercial and research activities, clearly deal with significant masses of infectious agents that are reasonably considered "production quantities."

However, in terms of potentially increased risk as a function of the mass of infectious agents, it is not possible to define "production quantities" in finite volumes or concentrations for any given agent. Therefore, the laboratory supervisor must make a risk assessment of the activities conducted and select practices, containment equipment, and facilities appropriate to the risk, irrespective of the volume or concentration of agent involved.

Occasions will arise when the laboratory supervisor should select a biosafety level higher than that recommended. For example, a higher biosafety level may be indicated by the unique nature of the proposed activity, e.g., the need for special containment for experimentally generated aerosols for inhalation studies or by the proximity of the laboratory to areas of special concern (e.g., a diagnostic laboratory located near patient care areas). Similarly, a recommended biosafety level may be adapted to compensate for the absence of certain recommended safeguards.

For example, in those situations when Biosafety Level 3 is recommended, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations, provided the recommended "Standard Microbiological Practices" and "Containment Equipment" for Biosafety Level 3 are rigorously followed.

The decision to adapt Biosafety Level 3 recommendations in this manner should be made only by the laboratory supervisor. This adaptation, however, is not suggested for agent

production operations or activities where procedures are frequently changing. The laboratory supervisor should also give special consideration to selecting appropriate safeguards for materials that may contain a suspected agent. For example, sera of human origin may contain hepatitis B virus and should be handled under conditions that reasonably preclude cutaneous, mucous membrane or parenteral exposure; sputa submitted to the laboratory for assay for tubercle bacilli should be handled under conditions that reasonably preclude the generation of aerosols or that contain any aerosols that may be generated during the manipulation of clinical materials or cultures.

The infectious agents that meet the previously stated criteria are listed by category of agent on the following pages. To use these summaries, first locate the agent in the listing under the appropriate category of agent. Second, utilize the practices, safety equipment, and type of facilities recommended for working with clinical materials, cultures of infectious agents, or infected animals recommended in the agent summary statement.

The laboratory supervisor is also responsible for appropriate risk assessment of agents not included in the agent Summary Statements and for utilization of appropriate practices, containment equipment, and facilities for the agent used.

Risk Assessment

Risk assessment of laboratory activities involving the use of infectious microorganisms is ultimately a subjective process. Those risks associated with the agent, as well as with the activity to be conducted, must be considered in the assessment. The characteristics of infectious agents and the primary laboratory hazards of working with the agent are described generically for agents in Biosafety Levels 1-3 and specifically for individual agents or groups of agents on pages 5 through 20 of this publication.

Hepatitis B virus (HBv) is an appropriate model for illustrating the risk assessment process. HBv is among the most ubiquitous of human pathogens and most prevalent of laboratory-associated infections. The agent has been demonstrated in a variety of body secretions and excretions. Blood, saliva, and semen have been shown to be infectious. Natural transmission is associated with parenteral inoculation or with contamination of the broken skin or of mucous membranes with infectious body fluids. There is no evidence of airborne or interpersonal spread through casual contact. Prophylactic measures include the use of a licensed vaccine in high-risk groups and the use of hepatitis B immune globulin following overt exposure.

The primary risk of HBv infection in laboratory personnel is associated with accidental parenteral inoculation, exposure of the broken skin or mucous membranes of the eyes, nose, or mouth, or ingestion of infectious body fluids. These risks are typical of those described for Biosafety Level 2 agents and are addressed by using the recommended standard and special microbiological practices to minimize or eliminate these overt exposures.

Hepatitis non-A, non-B, and acquired immunodeficiency syndrome (AIDS) pose similar infection risks to laboratory personnel. The prudent practices recommended for HBv are applicable to these two disease entities, as well as to the routine laboratory manipulation of

clinical materials of domestic origin.

The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human disease. Microbiological studies of animal host-specific pathogens, soil, water, food, feeds, and other natural or manufactured materials by comparison, pose substantially lower risk of laboratory infection. Microbiologists and other scientists working with such materials may, nevertheless, find the practices, containment equipment, and facility recommendations described in this publication of value in developing operational standards to meet their own assessed needs.

**Rickettsial Agents**

DVBID does not routinely work with rickettsial agents. The following information is provided for quick reference in the event of an accidental introduction (e.g., feral ticks or clinical specimens.)

**Agent: *Coxiella burnetii***

Pike's[1] summary indicates that Q fever is the second most commonly reported laboratory-associated infection with outbreaks involving 15 or more persons recorded in several institutions. A broad range of domestic and wild mammals are natural hosts for Q fever and may serve as potential sources of infection for laboratory and animal care personnel. Exposure to naturally infected and often asymptomatic sheep and to their birth products is a documented hazard to personnel. The agent is remarkably resistant to drying and is stable under a variety of environmental conditions.

Laboratory Hazards

The agent may be present in infected arthropods and in the blood, urine, feces, milk, and tissues of infected animal or human hosts. The placenta of infected sheep may contain $10^9$ organisms per gram of tissue, and milk may contain $10^5$ organisms per gram. Parenteral inoculation and exposure to infectious aerosols and droplets are the most likely sources of infection to laboratory and animal care personnel. The estimated human ID $25_{50}$ (inhalation) for Q fever is 10 organisms.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. Biosafety Level 3 practices and facilities are recommended for **activities involving the inoculation, incubation, and harvesting of** embryonated eggs or tissue cultures, the necropsy of infected animals, and the manipulation of infected tissues. Since infected guinea pigs and other rodents may shed the organisms in urine or feces, experimentally infected rodents should be maintained under Animal Biosafety Level 3. Recommended precautions for facilities using sheep as experimental animals are described by Spinelli and by Bernard. An investigational new phase 1- Q fever vaccine (IND) is available from the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick, Maryland. The use of this vaccine should be limited to those at high risk of exposure who have no demonstrated sensitivity to Q fever antigen.

**Agent: *Rickettsia prowazekii*, *Rickettsia typhi (R. mooseri)*, *Rickettsia tsutsugamushi*, *Rickettsia canada*, and Spotted Fever Group agents of human disease other than *Rickettsia rickettsii* and *Rickettsia akari***

Pike[1] reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with 3 deaths, and 68 cases of murine typhus. More recently, 3 cases of murine typhus were reported from a research facility. Two of these 3 cases were associated with work with infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation. These 3 cases represented an attack rate of 20% in personnel working with infectious materials.

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[1]Pike, R.M. Health Lab Sci. **13**:105-114, 1976.

Laboratory Hazard

Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of laboratory-associated infections. Naturally or experimentally infected lice, fleas and flying squirrels *(Glaucomys* spp) may also be a direct source of infection to laboratory personnel. The organisms are relatively unstable under ambient environmental conditions.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures and for the staining of impression smears. Biosafety Level 3 practices and facilities are recommended for all other manipulations of known or potentially infectious materials, including necropsy of experimentally infected animals and trituration of their tissues and inoculation, incubation, and harvesting of embryonated eggs or tissue cultures. Animal Biosafety Level 2 practices and facilities are recommended for activities with infected mammals other than flying squirrels or arthropods. Vaccines are not currently available for use in humans. Because the mode of transmission of *Rickettsia prowazekii* from flying squirrels to humans is not defined, Animal Biosafety Level 3 practices and facilities are recommended for animal studies with flying squirrels naturally or experimentally infected with *R. prowazekii*.

**Agent: *Rickettsia rickettsii***

Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike[1] reported 63 laboratory-associated cases, 11 of which were fatal. Oster[2] reported 9 cases occurring over a 6-year period in one laboratory, which were believed to have been acquired as a result of exposure to infectious aerosols.

Laboratory Hazards

Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of laboratory-associated infection. Successful aerosol transmission has been experimentally documented in nonhuman primates naturally and experimentally. Infected mammals, their ectoparasites, and their infected tissues are sources of human infection. The organism is relatively unstable under ambient environmental conditions.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all nonpropagative laboratory procedures, including serological and fluorescent antibody tests and staining of impression smears. Biosafety Level 3 practices and facilities are recommended for all other manipulations of known or potentially infectious materials,

[1]Pike, R.M. Health Lab Sci. **13**:105-114, 1976.

[2]Oster C.N. et al. N Engl J Med. **297**:859-862, 1977.

including necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or tissue cultures. Animal Biosafety Level 2 practices and facilities are recommended for holding of experimentally infected rodents; however, necropsy and any subsequent manipulation of tissues from infected animals should be conducted at Biosafety Level 3.

Because of the proven value of antibiotic therapy in the early stages of infection, it is essential that laboratories working with *R. rickettsii* have an effective system for reporting febrile illnesses in laboratory personnel, medically evaluating potential cases, and, when indicated, instituting appropriate antibiotic therapy. Vaccines are not currently available for use in humans.

## Viral Agents

DVBID does not routinely work with the viral agents listed in this section. The information is provided as a quick reference in the event of an accidental introduction (e.g., clinical specimens.)

### Agent: Hepatitis A Virus

Laboratory-associated infections with hepatitis A virus do not *appear* to be an important occupational risk among laboratory personnel. However, the disease is a documented hazard in animal handlers and others working with chimpanzees that are naturally or experimentally infected.

#### Laboratory Hazards

The agent may be present in feces of infected humans and chimpanzees. Ingestion of feces, stool suspensions, and other contaminated materials is the primary hazard to laboratory personnel. The importance of aerosol exposure has not been demonstrated. Attenuated or avirulent strains have not been fully defined but appear to result from serial passage in tissue culture.

#### Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with known or potentially infected feces from humans or chimpanzees. Animal Biosafety Level 2 practices and facilities are recommended for activities using naturally or experimentally infected chimpanzees. Animal care personnel should wear gloves and take other appropriate precautions to avoid possible fecal-oral exposure. Vaccines are not available for use in humans, but are in the developmental stages.

### Agent: Hepatitis B, Hepatitis non-A, non-B

Pike[1] concluded that hepatitis B is currently the most frequent laboratory-associated

[1]Pike, R.M. Health Lab Sci. **13**:105-114, 1976.

infection. The incidence in some categories of laboratory workers is seven times greater than that of the general population. Epidemiological evidence indicates that hepatitis non-A, non-B is a blood-borne disease similar to hepatitis B.

Laboratory Hazard

The agent of hepatitis B may be present in blood and blood products of human origin, in urine, semen, cerebrospinal fluid, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards. The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains are not defined.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other nonhuman primates. Gloves should be worn when working with infected animals and when there is the likelihood of skin contact with infectious materials. A licensed inactivated vaccine is available and is recommended for laboratory personnel who are at substantially greater risk of acquiring infection than the general population.

**Agent: Herpes virus simiae (B-virus)**

Although B-virus presents a potential hazard to laboratory personnel working with the agent, laboratory-associated human infections with B-virus have, with rare exceptions, been limited to personnel having direct contact with living Old World monkeys. Exposure to *in vitro* monkey tissues (i.e., primary rhesus monkey kidney) has been associated with a single documented case.

B-virus is an indigenous chronic and/or recurrent infection of macaques and possibly other Old World monkeys and is a frequent enzootic infection of captive *Macaca mulatta*.

Laboratory personnel handling Old World monkeys run the risk of acquiring B-virus from a bite or contamination of broken skin or mucous membranes by an infected monkey. Fifteen fatal cases of human infections with B-virus have been reported.

Laboratory Hazards

The agent may be present in oral secretions, thoracic, and abdominal viscera, and central nervous system tissues of naturally infected macaques. Bites from monkeys with oral herpes lesions are the greatest hazard to laboratory and animal care personnel. Exposures of broken skin or mucous membranes to oral secretions or to infectious culture fluids are also potential hazards. The importance of aerosol exposure is not known. Attenuated or avirulent strains

have not been defined.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of tissues, body fluids, and primary tissue culture materials from macaques. Additional containment and personnel precautions, such as those recommended for Biosafety Level 3, are recommended for activities involving the use or manipulation of any material known to contain *Herpesvirus simiae*.

Biosafety Level 4 practices, containment equipment, and facilities are recommended for activities involving the propagation of *H. simiae,* manipulations of production quantities or concentrations of *H. simiae,* and when housing vertebrate animals with proven natural or induced infection with the agent.

The wearing of gloves, masks, and laboratory coats is recommended for all personnel working with nonhuman primates -- especially macaques and other Old World species -- and for all persons entering animal rooms where nonhuman primates are housed. Vaccines are not available for use in humans.

**Agent: Herpesviruses**

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. While these viruses are not demonstrated causes of laboratory-associated infections, they are primary, as well as opportunistic, pathogens--especially in immuno-compromised hosts. Nonpolio enteroviruses, adenoviruses, and cytomegalovirus pose similar low potential infection risks to laboratory personnel. Although this diverse group of indigenous viral agents does not meet the criteria for inclusion in agent-specific summary statements (i.e., demonstrated or high potential hazard for laboratory-associated infection; grave consequences should infection occur), the frequency of their presence in clinical materials and their common use in research warrants their inclusion in this publication.

Laboratory Hazards

Clinical materials and isolates of herpesviruses, nonpolio enteroviruses, and other indigenous pathogens may pose a risk of infection following ingestion; accidental parenteral inoculation; droplet exposure of the mucous membranes of the eye, nose, or mouth; or inhalation of concentrated aerosolized materials.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as primary pathogens of human disease. Although there is no definitive evidence that infectious aerosols are a significant source of laboratory-associated infections, it is prudent to avoid the generation of aerosols during the

handling of clinical materials, isolates, or during the necropsy of animals. Primary containment devices (e.g., biological safety cabinets) constitute the basic barrier protecting personnel from exposure to infectious aerosols.

**Agent: Lymphocytic Choriomeningitis (LCM) Virus**

Laboratory-associated infections with LCM virus are well documented in facilities where infections occur in laboratory rodents -- especially mice and hamsters. Tissue cultures that have inadvertently become infected represent a potential source of infection and dissemination of the agent. Natural infections are occasionally found in nonhuman primates, swine, and dogs.

Laboratory Hazards

The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces, and tissues of infected humans and other animal hosts. Parenteral inoculation, inhalation, contamination of mucous membranes or broken skin with infectious tissues, or fluids from infected animals are common hazards. Aerosol transmission is well documented.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids or tissues and for tissue culture passage of mouse brain-passaged strains. All manipulations of known or potentially infectious passage and clinical materials should be conducted in a biological safety cabinet. Additional primary containment and personnel precautions such as those described for Biosafety Level 3 may be indicated for activities with high potential for aerosol production and for activities involving production quantities or concentrations of infectious materials. Animal Biosafety Level 2 practices and facilities are recommended for studies in adult mice with mouse brain-passaged strains. Animal Biosafety Level 3 practices and facilities are recommended for work with infected hamsters. Vaccines are not available for use in humans.

**Agent: Poxviruses**

Sporadic cases of laboratory-associated poxvirus infections have been reported. Pike lists 24 cases of yaba and tanapox virus infection and 18 vaccinia and smallpox infections. Epidemiological evidence suggests that transmission of monkeypox virus from nonhuman primates or rodents to humans may have occurred in nature but not in the laboratory setting. Naturally or experimentally infected laboratory animals are a potential source of infection to exposed unvaccinated laboratory personnel.

Laboratory Hazards

The agents may be present in lesion fluids or crusts, respiratory secretions, or tissues of infected hosts. Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues are the primary hazards to

laboratory and animal care personnel. Some poxviruses are stable at ambient temperature when dried and may be transmitted by fomites.

Recommended Precautions

The possession and use of variola viruses is restricted to the World Health Organization Collaborating Center for Reference and Research on Smallpox and Other Poxviruses Infections located at the Centers for Disease Control, Atlanta, Georgia. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of poxviruses other than variola that pose an infection hazard to humans. All persons working in or entering laboratory or animal care areas where activities with vaccinia, monkeypox, or cowpox viruses are being conducted should have documented evidence of satisfactory vaccination within the preceding three years. Activities with vaccinia, cowpox, or monkeypox viruses in quantities or concentrations greater than those present in diagnostic cultures may also be conducted by immunized personnel at Biosafety Level 2, provided that all manipulations of viable materials are conducted in Class I or II biological safety cabinets or other primary containment equipment.

**Agent: Rabies Virus**

Laboratory-associated infections are extremely rare. Two have been documented. Both presumably resulted from exposure to infectious aerosols of high titer generated in a vaccine production facility and a research facility. Naturally or experimentally infected animals, their tissues, and their excretions are potential sources of exposure to laboratory and animal care personnel.

Laboratory Hazards

The agent may be present in all tissues of infected animals. Highest titers are demonstrated in central nervous system tissue, salivary glands, and saliva. Accidental parenteral inoculation, cuts, or sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious droplets of tissue or fluids are the most likely sources of exposure for laboratory and animal care personnel. Infectious aerosols have not been a demonstrated hazard to personnel working with clinical materials and conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the only two recorded cases of laboratory-associated rabies resulted from exposure to a fixed Challenge Virus Standard (CVS) and an attenuated strain derived from SAD (Street Alabama Dufferin) strain.

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials. Preexposure vaccination is recommended for all individuals working with infected animals or engaged in diagnostic, production, or research activities with rabies virus. Preexposure vaccination is also recommended for all individuals entering or working in the same room where rabies virus or infected animals are used. While it is not feasible to open the skull or remove the brain

within a biological safety cabinet, it is pertinent to wear heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments and to wear a face shield to protect the mucous membranes of the eyes, nose, and mouth from exposure to infectious droplets or tissue fragments. If a Stryker saw is used to open the skull, avoid striking the brain with the blade of the saw. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with a high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

**Agent: Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob and Kuru Agents)**

Laboratory-associated infections with the transmissible spongiform encephalopathies have not been documented. The consequences of infection are grave, however, and there is evidence that Creutzfeldt-Jakob disease (CJD) has been transmitted to patients by corneal transplant and by contaminated electroencephalographic electrodes. There is no known nonhuman reservoir for CJD or kuru. Nonhuman primates and other laboratory animals have been infected by inoculation, but there is no evidence of secondary transmission.

Laboratory Hazards

High titers of a transmissible agent have been demonstrated in the brain and spinal cord of persons with kuru. In persons with Creutzfeldt-Jakob disease, a transmissible agent has been demonstrated in the brain, spleen, liver, lymph nodes, lungs, spinal cord, kidneys, cornea, and lens. Accidental parenteral inoculation is especially hazardous. Although nonneural tissues are less often infective, all tissues of humans and animals infected with these agents should be considered potentially hazardous. The risk of infection from aerosols, droplets, and exposure to intact skin, gastric mucous membranes is not known; however, there is no evidence of contact or aerosol transmission. These agents are characterized by extreme resistance to conventional inactivation procedures, including irradiation, boiling, and chemicals (formalin, betapropiolactone, alcohols).

Recommended Precautions

Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals. Extreme care must be taken to avoid accidental autoinoculation or other traumatic parenteral inoculations of infectious tissues and fluids. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues, fluids, and during the necropsy of experimental animals. It is further recommended that gloves should be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids. Vaccines are not available for use in humans.

**Agent: Vesicular Stomatitis Virus (VSV)**

Forty-six laboratory-associated infections with indigenous strains of VSV have been

reported. Laboratory activities with indigenous strains of VSV present two different levels of risk to laboratory personnel and are related, at least in part, to the passage history of the strains utilized. Activities utilizing infected livestock, their infected tissues, and virulent isolates from these sources are a demonstrated hazard to laboratory and animal care personnel. Seroconversion and clinical illness rates in personnel working with these materials are high. Similar risks may be associated with exotic strains such as Piry.

In contrast, anecdotal information indicates that activities with less virulent laboratory-adapted strains (e.g., VSV-Indiana [San Juan and Glasgow]) are rarely associated with seroconversion or illness. Such strains are commonly used by molecular biologists, often in large volumes and high concentrations, under conditions of minimal or no primary containment. Experimentally infected mice have not been a documented source of human infection.

Laboratory Hazards

The agent may be present in vesicular fluid, tissues, and blood of infected animals and in blood and throat secretions of infected humans. Exposure to infectious aerosols, infected droplets, direct skin and mucous membrane contact with infectious tissues and fluids, and accidental autoinoculation are the primary laboratory hazards associated with virulent isolates. Accidental parenteral inoculation and exposure to infectious aerosols represent potential risks to personnel working with less virulent laboratory-adapted strains.

Recommended Precautions

Biosafety Level 3 practices, containment equipment, and facilities are recommended for activities involving the use or manipulation of infected tissues and virulent isolates from naturally or experimentally infected livestock. Gloves and respiratory protection are recommended for the necropsy and handling of infected animals. Biosafety Level 2 practices and facilities are recommended for activities utilizing laboratory-adapted strains of demonstrated low virulence. Vaccines are not available for use in humans.

**Arboviruses Assigned to Biosafety Level 2**

The American Committee on Arthropod-Borne Viruses (ACAV) registered 424 arboviruses as of December 31, 1979. The ACAV's Subcommittee on Arbovirus Laboratory Safety (SALS) has categorized each of these 424 agents into 1 of 4 recommended levels of practice and containment which parallel the recommended practices, safety equipment, and facilities described in this publication as Biosafety Levels 1-4. It is the intent of SALS to periodically update the 1980 publication by providing a supplemental listing and recommended levels of practice and containment for arboviruses registered since 1979. SALS categorizations were based on risk assessments from information provided by a worldwide survey of 585 laboratories working with arboviruses. SALS recommended that work with the majority of these agents should be conducted at the equivalent of Biosafety Level 2. These viruses are listed alphabetically on pages 33 through 36 and include the following agents which are the reported cause of laboratory-associated infections. The list of arboviruses in Biosafety Level 2 includes yellow fever virus (17D strain) and Venezuelan equine encephalomyelitis (VEE)

virus (TC83 strain), provided that personnel working with these vaccine strains are immunized.

| Virus | | Cases (SALS) |
|---|---|---|
| Vesicular stomatitis | | 46 |
| Colorado tick fever | | 16 |
| Dengue | | 11 |
| Pichinde | | 17 |
| Western equine encephalomyelitis | (2 deaths) | 7 |
| Rio Bravo | | 7 |
| Kunjin | | 6 |
| Catu | | 6 |
| Caraparu | | 5 |
| Ross River | | 5 |
| Bunyamwera | | 4 |
| Eastern equine encephalomyelitis | | 4 |
| Zika | | 4 |
| Apeu | | 2 |
| Marituba | | 2 |
| Tacaribe | | 2 |
| Muructucu | | 1 |
| O'nyong-nyong | | 1 |
| Modoc | | 1 |
| Oriboca | | 1 |
| Ossa | | 1 |
| Keystone | | 1 |
| Bebaru | | 1 |
| Bluetongue | | 1 |

The results of the SALS survey clearly indicate that the suspected source of the laboratory-associated infections listed above was something other than infectious aerosols. Recommendations that work with these 334 arboviruses should be conducted at Biosafety Level 2 was based on the existence of adequate historical laboratory experience to assess risks for the virus which indicated that: a) no overt laboratory-associated infections are reported, b) infections resulted from exposures other than to infectious aerosols, or c) if aerosol exposures are documented, they represent an uncommon route of exposure.

Laboratory Hazard

Agents listed in this group may be present in blood, CSF, the central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection. While the primary laboratory hazards are accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods, infectious aerosols may also be a potential source of infection.

Recommended Precautions

Biosafety Level 2 practices, safety equipment, and facilities are recommended for activities with potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonated eggs, and rodents. Infection of newly hatched chickens with

eastern and western equine encephalomyelitis viruses is especially hazardous and should be undertaken under Biosafety Level 3 conditions by immunized personnel. Investigational vaccines (IND) against eastern equine encephalomyelitis and western equine encephalomyelitis viruses are available through the CDC. The use of these vaccines is recommended for personnel who work directly and regularly with these two agents in the laboratory. Western equine encephalomyelitis immune globulin (human) is also available from the CDC. The efficacy of this product has not been established.

Arboviruses Assigned to Biosafety Level 2

Abras
Abu Hammad
Acado
Acara
Aguacate
Alfuy
Almpiwar
Amapari
Ananindeua
Anhanga
Anhembi
Anopheles B
Anopheles A
Apeu
Apoi
Aride
Arkonam
Aroa
Aruac
Arumowat
Aura
Avalon
Babahoyo
Bagaza
Bahig
Bakau
Baku
Bandia
Bangoran
Bangui
Banzi
Barmah Forest
Barur
Batai
Batama
Bauline
Bebaru
Belmont
Benevides
Benfica
Bertioga
Bimiti
Birao
Bluetongue (indigenous)
Boraceia
Botambi
Boteke
Bouboui
Bujaru
Bunyamwera
Bunyip Creek
Burg el Arab
Bushbush
Bussuquara
Buttonwillow
Bwamba
Cacao
Cache Valley
Caimito
California encephalitis
Calovo
Candiru
Cape Wrath
Capim
Caraparu
Carey Island
Catu
Chaco
Chagres
Chandipura
Changuinola
Charleville
Chenuda
Chilibre
Chobar Gorge
Clo Mor
Colorado tick fever
Corriparta
Cotia
Cowbone Ridge
Csiro Village
Cuiaba
D'Aguilar
Dakar bat
Dengue-1
Dengue-2
Dengue-3
Dengue-4
Dera Ghazi Khan
Eastern equine encephalitis

Edge Hill
Encephalitis
Entebbe bat
Epidemic hemorraghic disease
Eubenangee
Eyach
Flanders
Fort Morgan
Frijoles
Gamboa
Gan Gan
Gomoka
Gossas
Grand Arbraud
Great Island
Guajara
Guama
Guaratuba
Guaroa
Gumbo Limbo
Hart Park
Hazara
Highlands J
Huacho
Hughes
Icoaraci
Ieri
Ilesha
Ilheus
Ingwavuma
Inkoo
Ippy
Irituia
Isfahan
Itaporanga
Itaqui
Jamestown Canyon
Japanaut
Jerry Slough
Johnstown Atoll
Joinjakaka
Juan Diaz
Jugra
Jurona
Jutiapa
Kadam
Kaeng Khoi
Kaikalur
Kaisodi
Kamese
Kammavanpettai
Kannamangalam
Kao Shuan
Karahi
Karimabad
Kasba
Kemerovo
Kern Canyon
Ketapang
Keterah
Keuraliba
Keystone
Klamath
Kokobera
Kolongo
Koongol
Kotonkan
Kovanyama
Kunjin
Kununurra
Kwatta
La Crosse
La Joya
Lagos bat
Landjia
Langat
Lanjan
Las Maloyas
Latino
Le Dantec
Lebombo
Lednice
Lipovnik
Lokern
Lone Star
Lukuni
M'Poko
Madrid
Maguari
Mahogany Hammock
Main Drain
Malakal
Manawa

Manzanilla
Mapputta
Maprik
Marco
Marituba
Marrakai
Matariya
Matruh
Matucare
Melao
Mermet
Minatitlan
Mitchell River
Modoc
Moju
Mono Lake
Montana Myotis
Leukoencephalitis
Moriche
Mossuril
Mount Elgon bat
Murutucu
Mykines
Navarro
Nepuyo
Ngaingan
Ninnal
Nique
Nirim
Nkolbisson
Nola
Ntaya
Nugget
Nyamanini
Nyando
O'Nyong-Nyong
Okhotskiy
Okola
Olifantsvlei
Oriboca
Ossa
Pacora
Pacui
Pahayokee
Palyam
Paramushir
Parana
Pata
Pathum Thani
Patois
Phnom-Penh bat
Pichinde
Pixuna
Pongola
Ponteves
Precarious Point
Pretoria
Prospect Hill
Puchong
Punta Salinas
Punta Toro
Qualyub
Quaranfil
Restan
Rio Bravo
Rio Grande
Ross River
Royal Farm
Sabo
Saboya
Saint-Floris
Sakhalin
Salehabad
San Angelo
Sandfly F. (Naples)
Sandfly F. (Sicilian)
Sandjimba
Sango
Sathuperi
Sawgrass
Seletar
Sembalam
Serra Do Navio
Shamonda
Shark River
Shuni
Silverwater
Simbu
Simian hemorrhagic fever
Sindbis
Sixgun City
Snowshoe Hare
Sokuluk
Soldado

Sororoca
Stratford
Sunday Canyon
Tacaiuma
Tacaribe
Taggert
Tahyna
Tamiami
Tanga
Tanjong Rabok
Tataguine
Tehran
Tembe
Tembusu
Tensaw
Tete
Tettnang
Thimiri
Thottapalayam
Tibrogargan
Timbo
Timboteua
Tindholmur
Toscana
Toure
Tribec
Triniti
Trivittatus
Trubanaman
Tsuruse
Turlock
Tyuleniy
Uganda S
Umatilla
Umbre
Una
Upolu
Urucuri
Usutu
Uukuniemi
Vellore
Venkatapuram
Vinces
Virgin River
VS-Indiana
VS-New Jersey
Wad Medani
Wallal
Wanowrie
Warrego
Western equine Whataroa
Witwatersrand
Wongal
Wongorr
Wyeomyia
Yaquina Head
Yata
Yogue
Zaliv Terpeniya
Zegla
Zika
Zingilamo
Zirqa

**Arboviruses and Arenaviruses Assigned to Biosafety Level 3**

SALS has recommended that work with the arboviruses included in the alphabetical listing on pages 39-40 be conducted at the equivalent of Biosafety Level 3 practices, safety equipment, and facilities. These recommendations are based on one of the following criteria: overt laboratory-associated infections with these agents have occurred by aerosol route if protective vaccines were not used or were unavailable; laboratory experience with the agent is inadequate to assess risk and the natural disease in humans is potentially severe, life threatening, or causes residual damage. Hantaan virus, which was not included in the SALS publication, has been placed at Biosafety Level 3, based on documented laboratory-associated infections. Rift Valley Fever virus, which was classified by SALS at Containment Level 3 (i.e., HFPA filtration required for all air exhausted from the laboratory), was placed in Biosafety Level 3 provided that all personnel entering the laboratory or animal care area where work with this virus is being conducted are vaccinated. Laboratory or laboratory animal-associated infections have been reported with the following agents:

| **Virus** | **Cases (SALS)** | |
|---|---|---|
| Venezuelan equine encephalitis | 18O | (1 death) |
| Rift Valley fever | 47 | (1 death) |
| Chikungunya | 39 | |
| Yellow fever | 38 | (8 deaths) |
| Japanese encephalitis | 22 | |
| Louping ill | 22 | |
| West Nile | 18 | |
| Lymphocytic choriomeningitis | 15 | |
| Orungo | 13 | |
| Piry | 13 | |
| Wesselsbron | 13 | |
| Mucambo | 10 | |
| Oropouche | 7 | |
| Germiston | 6 | |
| Bhanja | 6 | |
| Hantaan (Korean hemorrhagic fever) | 6 | |
| Mayaro | S | |
| Spondweni | 4 | |
| St. Louis encephalitis | 4 | |
| Murray Valley encephalitis | 3 | |
| Semliki Forest | 3 | (1 death) |
| Powassan | 2 | |
| Dugbe | 2 | |
| Issyk-kul | | |
| Koutango | | |

Large quantities and high concentrations of Semliki Forest virus are commonly used or manipulated by molecular biologists under conditions of moderate or low containment. Although antibodies have been demonstrated in individuals working with this virus, the first overt (and fatal) laboratory-associated infection with this virus was reported in 1979. Because this infection may have been influenced by a compromised host, and unusual route of exposure or high dosage, or a mutated strain of the virus, this case and its outcome may not be typical. Since exposure to an infectious aerosol was not indicated as the probable mode of transmission in this case, it is suggested that most activities with Semliki Forest disease virus can be safely conducted at Biosafety Level 2.

Some viruses (e.g., Ibaraki, Israel turkey meningoencephalitis) are listed by SALS in Level 3, not because they pose a threat to human health, but because they are exotic diseases of domestic livestock or poultry.

Laboratory Hazards

The agents listed in this group may be present in blood, CSF, urine, and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and broken skin contact. Some of these agents (e.g., VEE) may be relatively stable in dried blood or exudates. Attenuated strains are identified in a number of the agents listed (e.g., yellow fever-17D strain and VEE-TC83 strain).

Recommended Precautions

Biosafety Level 3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

A licensed attenuated live virus vaccine is available for vaccination against yellow fever and is recommended for all personnel who work with this agent or with infected animals and those who enter rooms where the agents or infected animals are present. An investigational vaccine (IND) is available for immunization against VEE and is recommended for all personnel working with VEE (and the related Everglades, Mucambo, Tonate, and Cabassou viruses), infected animals, or entering rooms where these agents or infected animals are present. Work with Hantaan (Korean hemorrhagic fever) virus in rats, voles, and other laboratory rodents should be conducted with special caution (Biosafety Level 4). An inactivated, investigational new Rift Valley fever vaccine (IND) is available from the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) and is recommended for all laboratory and animal care personnel working with the agent or infected animals and for all personnel entering laboratories or animal rooms when the agent is in use.

Arboviruses Assigned to Biosafety Level 3

Adelaide River
Agua Preta
Aino
Akabane
Alenquer
Almeirim
Altamira
Antequera
Araguari
Aransas Bay
Arbia
Barranqueras
Batken
Belem
Berrimah
Bhanja
Bimbo
Bobaya
Bobia
Buenaventura
Cabassou[b]
Cacipacore
Calchaqui
Cananeia
Caninde
Chikungunya[b]
Chim
Coastal Plains
Cocal
Connecticut
Dhori
Douglas
Dugbe
Enseada
Estero Real
Everglades[b]
Flexal
Gadgets Gully
Garba
Germiston[b]
Getah
Gordil
Gray Lodge
Gurupi
Hantaan
Iaco
Ibaraki
Ife
Inhangapi
Inini
Israel turkey men.
Issyk-Kul
Itaituba
Itimirim
Itupiranga
Jacareacanga
Jamanxi
Japanese enceph.
Jari
Kairi
Khasan
Kimberley
Kindia
Koutango
Kyzylagach
Lake Clarendon
Llano Seco
Macaua
Mapuera
Mayaro
Meaban
Middelburg
Mojui Dos Campos
Monte Dourado
Mucambo[b]
Munguba
Murray Valley Enceph.
Naranjal
Nariva
Ndumu
Negishi
New Minto
Nodamura
Northway
Oriximina
Oropouche[b]
Orungo
Ouango
Oubangui
Ourem

Palestina
Para
Paramushir
Paroo River
Peaton
Picola
Piry
Playas
Powassan
Pueblo Viejo
Purus
Razdan
Resistencia
Rift Valley Fever[a,b]
Rochambeau
Rocio[b]
Sagiyama
Salanga
Sal Vieja
San Juan
San Perlita
Santarem
Santa Rosa
Saraca
Saumarez Reef
Semliki Forest
Sena Madureira
Sepik
Shokwe
Spondweni
Sripur
St. Louis encephalitis
Tamdy
Telok Forest
Termeil
Thogoto
Tilligerry
Tinaroo
Tlacotalpan
Tonate[b]
Turuna
Utinga
Venezuelan equine encephalitis[b]
VS-Alagoas
Wesselbron[a,b]
West Nile
Xiburema
Yacaaba
Yellow Fever[b]
Yug Bogdanovac
Zinga

a) The importation, possession, or use of this agent is restricted by USDA regulation or administrative policy.

b) SALS recommends that work with this agent be conducted only in Biosafety Level 3 facilities, which provide for HEPA filtration of all exhaust air prior to discharge from the laboratory. All persons working with agents for which a vaccine is available should be vaccinated.

**Arboviruses, Arenaviruses, or Filoviruses Assigned to Biosafety Level 4**

SALS has recommended that work with the arboviruses, arenaviruses, or filoviruses included in the listing that follows should be conducted at the equivalent of Biosafety Level 4 practices, safety equipment, and facilities. These recommendations are based on documented cases of severe and frequently fatal naturally occurring human infections and aerosol-transmitted laboratory infections. Additionally, SALS recommended that certain agents with a close or identical antigenic relationship to the Biosafety Level 4 agents (e.g., Absettarov and Kumlinge viruses) also be handled at this level until sufficient laboratory experience is obtained to retain these agents at this level or to work with them at a lower level. Laboratory or laboratory animal-associated infections have been reported with the following agents.

| **Virus** | **Cases (SALS)** | |
|---|---|---|
| Kyasanur Forest disease | 133 | |
| Hypr | 37 | (2 deaths) |
| Junin | 21 | (1 death) |
| Marburg | 25 | (5 deaths) |
| Russian spring-summer encephalitis | 8 | |
| Congo-Crimean hemorrhagic fever | 8 | (1 death) |
| Omsk hemorrhagic fever | 5 | |
| Lassa | 2 | (1 death) |
| Machupo | 1 | (1 death) |
| Ebola | | |

Rodents are natural reservoirs of Lassa fever virus *(Mastomys natalensis*), Junin and Machupo viruses *(Calomys* spp.) and perhaps other viruses assigned to Biosafety Level 4. Nonhuman primates were associated with the initial outbreaks of Kyasanur Forest disease *(Presbutis* spp.) and Marburg disease *(Cercopithecus* spp.), and arthropods are the natural vectors of the tick-borne encephalitis complex agents. Work with or exposure to rodents, nonhuman primates, or vectors naturally or experimentally infected with these agents represents a potential source of human infection.

Laboratory Hazards

The infectious agents may be present in blood, urine, respiratory and throat secretions, semen and tissues from human or animal hosts, and in arthropods, rodents, and nonhuman primates. Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel.

Recommended Precautions

Biosafety Level 4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a Biosafety Level 4 maximum containment facility.

Arboviruses, Arenaviruses, and Filoviruses Assigned to Biosafety Level 4

Congo-Crimean hemorrhagic fever
Tick-borne encephalitis virus complex
(Absettarov, Hanzalova, Hypr,
Kumlinge, Kyasanur Forest disease,
Omsk hemorrhagic fever, and
Russian spring-summer encephalitis)
Marburg
Ebola
Junin
Lassa
Machupo
Guaranito

Arboviruses Assigned to Biosafety Level 4

Absettarov
Congo-Crimean hemorrhagic fever
Ebola
Hanzalova
Hypr
Junin
Kumlinge
Kyasanur Forest disease
Machupo
Marburg
Omsk hemorrhagic fever
Russian spring-summer encephalitis

**Bacterial Agents - Biosafety Levels**

| **Agent** | **Level 2** | **Level 3** |
|---|---|---|
| *Francisella tularensis* | Clinical material | Cultures and animal work |
| *Leptospira interrogans* | All activities | ___ |
| *Salmonella choleraesuis* | All activities | ___ |
| *Salmonella enteritidis* | All activities | ___ |
| *Shigella* spp. | All activities | ___ |
| *Campylobacter fetus-jejuni* | All activities | ___ |
| *Vibrio cholerae* | All activities | ___ |
| *Vibrio parahaemolyticus* | All activities | ___ |
| *Yersinia pestis* | Clinical materials | Activities with aerosol and cultures, potential antibiotic resistant strains, production quantities |
| *Rickettsia rickettsii* | Nonpropagative procedures--serology, incubation and harvesting of eggs or tissue culture. | Necropsy, trituration, inoculation, fluorescent antibody-stained smears. |
| *Borrelia burgdorferi* other *Borrelia* spp. | Clinical materials and cultures. | Activities with aerosol potential, antibiotic-resistant strains, production quantities ($\geq$ 10 l). |

# BIOSAFETY PRINCIPLES AND PRACTICE FOR SPECIFIC PROCEDURES

## BACTERIOLOGY LABORATORY

### General Safety Rules

Biohazard signs must be posted on all appropriate entrances.

Lab coats must be worn while performing laboratory functions, and lab coats will not be worn outside laboratory areas.

Storage of hazardous chemicals/biochemicals must only be in appropriate containers and storage areas. Disposal of excess or used materials must follow appropriate protocol.

Hands must be washed on completion of each lab chore and before leaving laboratory (most definitely before eating, drinking, or rubbing eyes, etc.).

All laboratory accidents must be reported promptly to the laboratory supervisor and biosafety officer.

All reagents, solutions, cultures, specimens, etc., must be fully and legibly labeled.

No person should perform extremely hazardous operations when alone.

### Serology

Original, undiluted specimens of human origin are considered infectious and are handled only with gloved hands. Using the laminar flow biosafety cabinet for handling these specimens is recommended. Use of safety cabinets with multiple users should be scheduled such that all hazardous procedures can be accomplished in the safest way. Specimens should be stored at -20˚C or lower in intact, screw-capped, labeled tubes.

Inactivation and adsorption procedures should be done in capped tubes.

Centrifugation of specimens should be done only in intact tubes certified for speeds/forces to be used. Vortex mixing is done only in closed tubes in a biosafety cabinet.

Centrifuged and/or sonicated specimens are allowed to equilibrate before being opened (to prevent release of possible aerosols) or are opened in a biosafety cabinet.

"Decanting" is done by aspiration rather than pouring. Class II agents can be decanted outside a safety cabinet.

Used materials are decontaminated before leaving the laboratory. Used glassware, pipettes, syringes, needles, etc., are autoclaved or placed temporarily in a flat pan containing sufficient germicide to cover the contaminated items until they are autoclaved.

All used items are soaked in germicide for a period sufficient to ensure sterility. Disposable items are autoclaved before they are removed from the laboratory.

Countertops should be disinfected at the end of each procedure before staff members leave the work area.

A noncorrosive disinfectant in germicidal strength should be added to water baths.

In-house vacuum lines used for aspiration of potentially infectious materials should be fitted with suitable multiple traps to ensure that no infectious material enters the system. The primary trap should contain a disinfectant and the final trap should contain a desiccant.

**Microscopic Examination of Culture or Tissue Specimens**

Viable cultures must be considered infectious even after drying on microscope slides until fixed either by heat (adequate to kill bacteria) or chemical (alcohol, acetone, bleach, etc.) treatment. Slides for microscopic examination should be prepared in the biosafety hood.

Because cultures prepared for darkfield microscopic examination (e.g., spirochete cultures) are viable, slides must be handled as contaminated items; gloves should be worn while processing the slides. Once examination is completed, the slides will be placed in a pan containing disinfectant. Parts of the microscope that may have been contaminated should be cleaned with a germicidal solution (safe for microscope parts!) with gloved hands.

Smears of tissues to be examined for presence of bacteria should be considered as contaminated and handled with the same care given to viable cultures.

**Primary Bacterial Isolation**

All cultures, tissue, or fluid specimens (CSF, blood, urine, feces, sputa, liver, spleen, etc.) should be considered infectious. All manipulations in the biosafety cabinet should be performed using gloved hands and aseptic technique. This will also ensure that the specimen remains clean and free of introduced contaminants.

Original culture or tissue materials should be stored in capped containers; when no longer needed, they should be sterilized by autoclaving and discarded.

Only cotton-plugged pipettes should be used in all transfers of cultures or culture suspensions.

Keep inoculated and transfer petri dishes or other inoculated solid media in leak-proof containers.

Transport, incubate, and store fluid or viable powdered infectious materials in nonbreakable, leak-proof, easily handled containers.

Immerse contaminated pipettes, applicators, syringes, needles, etc., in pans containing disinfectant. Allow contaminated items to remain in disinfectant for sufficient time to ensure destruction of pathogens.

Prepare and store hazardous reagents used in biochemical characterization of bacterial strains appropriately. Dispose of contaminated, used, or surplus reagents after chemical or physical sterilization.

Centrifuge viable culture suspensions in capped, intact centrifuge tubes certified for the speeds and forces to be used. The sealed head should be removed from the centrifuge and taken to a biosafety cabinet to be opened. Tubes should be decanted by aspiration in the safety cabinet, and discarded materials should be placed in decontamination pans within the safety cabinet.

Perform sonication (potential generator of aerosols) in a biosafety cabinet; operator must be gloved and masked.

Avoid filling centrifuge tubes to the degree that the rims become wetted by culture suspensions.

All inoculated media, plates, tubes, biochemical identification kit strips, filter sheets/membranes, etc., are decontaminated in an autoclave prior to disposal.

Filtrates from membrane filtration procedures must be considered contaminated. Membrane filters (either 0.8 µ, 0.45 µ, 0.22 µ, or other pore sizes) are inherently fragile and susceptible to tears and leaks.

Vortex mixing of culture suspensions should be done only within a biosafety cabinet; use only intact, screw-capped, sealed tubes. Opening the vortexed tube should either be delayed for a few minutes or it should be opened within the hood.

Flaming of loops, flasks, and other items should be done in such a fashion as to minimize, if not prevent, splatter.

All procedures involving handling large volumes of viable cultures should be performed in biosafety cabinets; operators should wear gloves, gowns, and masks.

Animals are necropsied in a biosafety cabinet; tissues and other specimens are collected aseptically and placed in a container with a lid (a ziplock plastic bag will suffice). Specimens are transported to the bacteriology laboratory in the closed container.

Necropsy instruments are either placed in instrument sterilizer and boiled for 20 minutes or in a pan of disinfectant after each use. No instrument is re-used prior to disinfection.

Inoculated animals are segregated by origin of specimen; when possible, animals infected with different agents will be housed in separate rooms. (Exception: animals inoculated with diagnostic specimens containing unknown pathogens.)

Inoculated animals are considered infectious and handling should be minimal. When necessary, animals are picked up by forceps or with gloved hands (double gloving when necessary - first surgical gloves, then heavy-duty gloves to prevent skin puncture by animal bite). Under no circumstances should inoculated animals be handled with bare hands.

Some bacterial strains may be passed in urine and feces; thus, bedding must be considered contaminated. Masks should be worn when cleaning or otherwise disturbing bedding.

**Antibody Production**

<u>In Animals</u>

All animal procedures will meet animal care standards, as applicable. Only humane handling techniques will be permitted; if needed, animals will be anesthetized prior to manipulations.

Animals that are free of ectoparasites and disease are procured from reliable sources. The animals are kept under observation for a quarantine period of not less than 2 weeks before being used. Serum specimens are obtained from all animals, and baseline antibody titers are checked prior to vaccination.

Inoculate as per immunization schedule of interest. Animal hair is shaved or thoroughly wetted with germicidal antiseptic before inoculation. If viable infectious organisms are being inoculated, aseptic techniques must be maintained to protect inoculum, animal, and personnel from contamination.

Serum specimens are drawn aseptically on specified schedules. Site of needle puncture is swabbed with antiseptic; sterile instruments are used to collect specimens. If the animal is to be exsanguinated, it must first be anesthetized.

Blood, sera, and other tissues and body fluids from animals inoculated with viable agents are considered infectious until culture or other tests prove their sterility.

In Hybridomas

Preparation and management of monoclonal antibody hybridomas follow standard safe animal handling procedures and tissue culture techniques.

Aseptic techniques in performing cell fusions and in subsequent cell culture manipulations are critical -- both to protect personnel and hybridoma lines.

**Invertebrate Inoculations (for recovery of pathogens from vectors)**

Collections are made per usual protocol:

- fleas are placed in 2% saline with 0.1% Tween 80 and stored in the refrigerator until they are identified and processed;
- ticks are placed in dry vials;
- mosquitoes are frozen until identified and processed.

After being identified and pooled, ectoparasites are placed in clean mortars or tissue grinders and triturated with a pestle (any saline is aspirated prior to trituration.) Dissection (after a brief washing in 5% sodium hypochlorite solution to remove gross contamination from the exoskeleton) of fleas and ticks should be performed within a drop of physiologic saline in a petri dish (separate dish for each arthropod or arthropod pool.)

Animal inoculations with ectoparasite pool triturates are done aseptically. (See section on safe needle-handling techniques.)

Inoculated animals, now considered infected, are caged appropriately and observed twice daily for recommended holding periods (21 days for plague/tularemia specimens.)

Handling after inoculation is kept to a minimum and done with forceps or gloved hands. Soiled bedding, excess food, etc., are sterilized before disposal. Cages and water bottles are handled according to current animal care policies.

Squash procedures are performed in a biosafety hood whenever possible. Personnel performing the procedures outside a biosafety cabinet environment should wear masks and make the squashes inside a container with smooth and even motions directed away from the operator (and from other persons in the room.) (Jerky motions create aerosols!!) Squash smears should be considered contaminated until after heat, acetone, or alcohol fixation. Used slides (unwanted) should be placed in a leak-proof container and autoclaved prior to disposal.

Sonication of arthropod suspensions should be performed only in a safety cabinet and in intact, closed tubes or vessels. Tubes of sonicated suspensions should be allowed to stand for several minutes before they are opened, or they should be opened in a biosafety cabinet.

**Inoculation of Invertebrates with Viable Bacterial Agents**

Only axenic invertebrates will be used for vector infection studies or vector efficiency studies.

Numbers of invertebrates used in each study will be monitored by count. All infected and uninfected individuals will be accounted for and all possible measures to prevent escape of infected individuals either into laboratory space or to the outside environment will be taken.

All inoculation procedures should be carried out in the insectary unless approved in advance by the Biosafety Officer.

Only such volumes of infectious materials as can be effectively managed by the species being studied will be introduced. Care will be taken that aerosolization of infectious suspensions will be minimized, if not eliminated. All inoculations of invertebrates of Class III will be done in a biosafety cabinet. Inoculated specimens will be considered infected and infectious. Infected arthropods will be caged in escape-proof containers and handled only after being anesthetized. All manipulations will be done with gloved hands (and masks when a danger of aerosolization exists). A mask must also be worn during inoculations.

At the end of each experiment, counts of invertebrates used will be made and shown to correspond with numbers initially brought and infected.

## ARBOVIRUS LABORATORIES

### Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples

a. Protective clothing

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

None

c. Hood use:

One should assume that specimens sent for virus isolation contain virus. Therefore, all manipulations with such specimens should be done using a safety cabinet (hood). Work in hoods is to be done using plastic-coated absorbent paper to contain accidental spills. All disposable and nondisposable materials should be sterilized in an autoclave after use.

d. Steps that potentially produce aerosols:

Homogenization of virus-containing materials usually produces bubbles (which contain virus) that, on bursting, release small quantities of aerosolized virus and other particles. Therefore, specimens should be homogenized in a closed container. The tip of instruments used to mix specimens (i.e., for dilution) should be kept below the surface of the fluid, so that aerosols are not produced. If a mechanical blender is used, the lid is to be kept on the blender until all chances of aerosol production (such as bursting bubbles) are minimized.

e. Inoculation injuries:

Personnel working with viruses for which vaccines are available should be administered those vaccines and their serum subsequently shown to contain antibodies to that virus. Mixing of specimens should be done without use of syringe needles or other sharp objects. However, syringe needles are used for animal inoculations and routine care should be taken to prevent accidental self-inoculations. Needles should not be removed from syringes. Covers should not be replaced on needles. Used syringes with needles attached should be placed in special autoclave containers with disinfectant solution.

**Virus Titrations**

a. Protective clothing:

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

None

c. Hood use:

All manipulations with viruses should be done using a safety cabinet (hood). Work in hoods is to be done using plastic-coated absorbent paper to contain accidental spills. All disposable and nondisposable materials should be sterilized in an autoclave after use.

d. Steps that potentially produce aerosols:

(See Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples above)

e. Inoculation injuries:

(See Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples above)

**Antigen Preparation for Serologic Tests and Immunogens**

a. Protective clothing:

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

One of the many steps needed for production of antigen (inactivated or noninactivated) is the addition of acetone. This is a highly explosive chemical. It should be disposed of by pouring the used acetone into a metal container specified for this purpose. Only people with complete knowledge of disposal methods should work with this reagent; acetone is <u>not</u> to be poured down a sink.

Many chemical and physical agents can be used to inactivate viruses. However, we find that beta-propiolactone is most satisfactory. Unfortunately, beta-propiolactone is a carcinogen. It should be handled only by explicitly authorized individuals.

c. Hood use:

All manipulations with infectious antigens should be done using a safety cabinet (hood). Work in hoods is to be done using plastic-coated absorbent paper to contain accidental spills. All disposable and non-disposable materials should be sterilized in an autoclave after use.

d. Steps that potentially produce aerosols:

(See Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples above)

e. Inoculation injuries:

(See Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples above)

**Preparation of Antibodies in Serum and Ascitic Fluids**

a. Protective clothing:

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

The sarcoma 180 cells used to raise ascites in mice and certain other animals inoculated with them are polyploid (malignant) cells and may grow locally in humans accidentally inoculated with them.

c. Hood use:

All manipulations with such specimens should be done using a safety cabinet (hood). Work in hoods is to be done using plastic-coated absorbent paper to contain accidental spills. All disposable and nondisposable materials should be sterilized in an autoclave after use.

d. Steps potentially producing aerosols:

One assumes that sera for antibodies do not contain virus because they are collected far beyond the expected length of viremia. However, one should not depend absolutely on this and should at least keep in mind that the material might be infectious.

e. Inoculation injuries:

(See Primary Virus Isolation from Serum and Cerebrospinal Fluid Samples above)

**Lyophilization**

a. Protective clothing:

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

None

c. Hood use:

None

d. Steps that potentially produce aerosols:

None

e. Inoculation injuries:

None

**Serologic Tests**

Enzye-linked Immunosorbent Assay (ELISA)
Hemagglutination Inhibition Test (HIT)
Complement Fixation Test (CFT)
Neutralization Test (NT)

a. Protective clothing:

Laboratory coat, vinyl or latex gloves (to cover sleeve opening).

b. Reagent toxicity or fire hazard:

None. Many substrates formerly used in ELISA tests are carcinogenic; however, we no longer use these chemicals.

c. Hood use:

All manipulations with such specimens should be done using a safety cabinet (hood). Work in hoods is to be done using a plastic-coated absorbent paper to contain accidental spills. All disposable and nondisposable materials should be sterilized in an autoclave after use.

d. Steps that potentially produce aerosols:

None, if usual techniques are applied.

e. Inoculation injuries:

None, because sharp-pointed instruments are not used.

**Preparation and Purification of Viruses**

Safety.

All work with infectious viruses must be performed only in a designated area. Centrifuge infectious fluids only in this designated area.

When working with infectious agents, post a sign outside the laboratory door. Indicate the infectious agent and any limitations on entry of personnel (such as "Vaccinated Personnel Only"). Excluding non-vaccinated personnel from only one or two of the designated rooms may be appropriate. Do not ignore posted caution signs.

All work with infectious agents must be performed within a laminar flow biosafety cabinet.

Enter the date, time of work, infectious agent(s), and your initials in the log book posted at each safety cabinet.

Gloves must be worn at all times while working with infectious virus. Remove gloves before leaving safety cabinet or virus work area.

Wear a laboratory coat that opens from the back, not the front.

Work in the hood on plastic-coated absorbent paper to contain accidental spills. Sterilize paper in an autoclave when finished with work.

Plan activities and supply needs ahead of time so that everything is available in the safety cabinet when needed.

Take precautions to prevent the formation of infectious aerosols. Work in a biosafety cabinet. Drain liquids from pipettes -- do not blow them out. Decant fluids cautiously down sides of tubes, bottles, and flasks, rather than pouring directly onto bottom of vessel. Aspirate fluids whenever possible.

Do not fill centrifuge bottles or tubes more than 80% of their full capacity with infectious fluids!! Overfilled bottles will leak during centrifugation.

Wear a face mask when raising the centrifuge lid after specimens have been centrifuged. Carry rotor to safety cabinet before opening. Remove bottles or tubes in the safety cabinet.

Discard contaminated or potentially contaminated disposable items in a biohazard bag or autoclave pan. Place recyclable items (glassware, for example) in a separate pan for autoclaving. Do not mix disposable and recyclable items. Pans must contain some water to prevent autoclaved items from sticking. To avoid production of aerosols, add water to pans before placing under safety cabinet, not after work is finished. Potentially contaminated glassware may be placed in a central collecting tub for autoclaving. Infectious fluids must always be kept in covered vessels before they are autoclaved.

Clean out the hood after your work is finished. Do not leave debris for the next person to clean up. A few items (flashlight and safety flask for working with gradients) should remain in the safety cabinet. Disinfect the safety cabinet surface with 10% sodium hypochlorite (bleach) after use.

Add water to contaminated centrifuge bottles (one-third full) before autoclaving. Add the water carefully from a carrying vessel under the hood. Leave the bottle lids loose. If lids are tightened, bottles will collapse during the autoclave cycle, and the bottles will have to be discarded.

Affix autoclave tape to all pans, tubs, bags, and single items to be autoclaved. Tape lid of autoclave pan to bottom portion at both ends. Remove old tape from clean autoclave pans and tubs before use. Contaminated items must not be brought into the kitchen area.

Clean up spills of infectious fluids immediately. See section on spills (page 81). Autoclave or disinfect (10% bleach for 24 hr) contaminated items. Immediately report spills outside of the hood (including contaminated rotor or centrifuge) to your supervisor.

For centrifuge safety, always do the following:

Balance opposing tubes exactly. Balancing "by eye" is not good enough!

Match the serial number of each swinging bucket to the identical serial number of the

corresponding rotor.

Place bucket in appropriate (by number) slot of rotor and spin bucket #2 opposite #5, #1 - #4, and #3 - #6.

If a rotor has been used long enough or is old enough to be derated for speed, be sure not to exceed the derated speed for centrifugation.

After each centrifugation, rinse the rotor buckets (inside and out) with distilled water and then air dry.

When necessary, clean rotor buckets with special Beckman detergent (Solution 555 rotor cleaning concentrate; diluted 1:5 in water), using Beckman brushes which have protectors to prevent scratching of anodized surfaces.

**Preparation of Virus Seeds**

In addition to the general safety steps listed above, please note the following:

Thaw vials of stock virus in the hood.

During the centrifugation step, do not fill centrifuge bottles to more than 80% of their capacity.

**Purification of Viruses**

In addition to the general safety steps listed above, please note the following:

When adding polyethylene glycol to supernatant medium and using a stir bar, cover the flask tightly with Parafilm and aluminum foil.

When using a vortex to break up aggregates in a virus suspension, do so in a hood.

Balancing of tubes for centrifugation of virus suspensions must be performed in a biosafety cabinet. Beakers used to hold the tubes for centrifugation should be autoclaved, and balance pan surfaces should be disinfected with 10% bleach.

When preparing to visualize the virus band after rate - zonal ultracentri- fugation, be certain that the ringstand clamp will hold the centrifuge tube securely before releasing your grip on the centrifuge tube.

**Gradients**

Follow the general safety steps listed above in "Safety" Section. Also see Ultracentrifuge Safety in Appendix A.

**Immunofluorescence Experiments**

Fluorescent antibody (FA) or Immunofluorescent antibody (IFA) is frequently used to detect viral and bacterial antigens. Suspected infected tissue or cell culture is first fixed with various organic solvents and then reacted with fluorescent dye-labeled antibody to verify the presence of antigen by use of a fluorescence microscope.

All guidelines for handling viral and bacterial infected material should be followed.

Wear a laboratory coat and disposable gloves.  Remove them before leaving the laboratory.

Prepare material to be examined in a biological safety cabinet. If solvent cooled below room temperature is preferred for fixation, bring a bucket containing ice or dry ice into the safety cabinet.

Place contaminated materials in leakproof, stainless steel pans filled with water. Separate disposable and washable items. Close these pans before removing them from the cabinet.

Sterilize contaminated materials in an autoclave.

Dispose of organic solvents properly. Acetone, the most commonly used solvent in our laboratory, should be collected in a glass bottle, stored in an appropriate location and disposed of in the designated 5-gallon metal container in the explosion-proof refrigerator in the glassware preparation room.

Protect your eyes from UV radiation. When using a fluorescence microscope, never look at the excitation light directly. Even when handling the specimen slides, be sure to look through the UV protective shade.

**Polyacrylamide Gel Electrophoresis**

Solutions and Gel Preparation

Acrylamide is a neurotoxin and is readily absorbed through skin and by inhalation.

Use commercially prepared liquid solutions rather than crystalline acrylamide, if possible. Acrylamide sublimates.

Wear protective clothing, gloves, and safety glasses when handling acrylamide solutions and pouring gels.

Avoid spills and use absorbent paper on laboratory countertops.

If acrylamide comes into contact with skin or eyes, flush with water.

Unused acrylamide solutions should be polymerized with TEMED before disposal in containers marked for hazardous waste. Concentrated stocks of acrylamide produce considerable heat during polymerization; they should be cooled on ice before TEMED is added.

Do not pour liquid acrylamide into sink drains.

**Electrophoresis**

Gels undergo electrophoresis at high voltage. Designate a work area with minimal traffic in and out of room for gel electrophoresis.

Cover buffer reservoirs during electrophoresis.

Do not crowd gel devices on counters. Avoid tangled power cords. Use care when dismantling an apparatus to avoid electrical shock and spilling radioactive buffer.

**Radiation**

Buffer from reservoirs (particularly from the lower reservoir) is moderately radioactive and should be removed carefully and disposed of in a sink drain. Run water in the sink for 3-5 minutes after discarding buffer.

Wear safety glasses when loading and dismantling radioactive gels. Use plastic shielding when necessary for preparative gels.

Radioactive acrylamide gels should be disposed of in the designated container.

**Use of Radionuclides**

Radioactive materials and radiation sources must be used in compliance with regulations of the State of Colorado and of the U.S. Nuclear Regulatory Commission. We order radionuclides with the approval of the CSU Health Physicist and under a radioactive materials license issued to Colorado State University. To become an authorized user of radioactive materials, you must submit a Radiation Health History form and a Radiation Project Description form to the CSU Health Physicist. As CDC employees, we must also comply with the codes of safe practice of the CDC. Consequently, both the Colorado State University Radiation Control Manual and the CDC Radiation Safety Manual are required reading for radiation workers. Copies of each manual are available from the Division Radiation Safety Officer (RSO).

The following is a brief overview of requirements. It is not a substitute for the detailed radiation control manuals:

1. Training. If employees plan to use more than one control unit of a radionuclide, they must first attend a substantial training course (CSU R400 or equivalent). An approved 1-week course is offered periodically by CDC. Visitors must provide evidence of previous training to the CSU Health Physicist before they may work "solo" with radionuclides.

One control unit defined

1000 µCuries $^{3}H$
100 µCuries $^{35}S$
100 µCuries $^{14}C$
10 µCuries $^{32}P$

2. General Information. $^{3}H$, $^{14}C$, and $^{35}S$ emit low-energy ß particles; $^{35}P$ emits high energy ß particles. In practice, this means that $^{3}H$, $^{14}C$, and $^{35}S$ can be shielded by very thin plastic (plastic gloves stop virtually all the radiation), and they have very short path lengths in air: 6 mm, 24 cm, and 30 cm, respectively. For $^{32}P$, however, at least 1 cm of Lucite or equivalent shielding is required. $^{32}P$ emissions have a maximum range of 7.20 m in air. Contamination with $^{3}H$ can only be detected by scintillation counting of swabs. $^{14}C$ and $^{35}S$ may be detected with a G-M tube monitor that has a wide window. Generally, we use wipe tests for these radionuclides as well. $^{32}P$ is efficiently detected by a G-M tube; trace contamination by $^{32}P$ may be assessed with swabs.

   $^{125}I$ emits gamma rays and poses special problems. If ingested, approximately one-third localizes in the thyroid. Lead ($\geq$0.25 mm) is needed for shielding. We do not currently have approval to order $^{125}I$.

3. Limits for occupational exposure (for person 18 years of age or over).

   Whole body; head and trunk; 1.25 rem/quarter
   active blood-forming organs;
   lens of eye; or gonads.

   Hands and forearms; feet and 18.75 rem/quarter
   ankles

   Skin of whole body 7.50 rem/quarter

   If necessary, the quarterly quota may be received as a single dose, but it is undesirable for doses of this magnitude to be repeated at close intervals.

   Prenatal exposure should not exceed a cumulative dose equivalent to 0.5 rem during the entire period of gestation. Staff who have reason to believe that they are pregnant should inform their supervisor in order that potential exposures can be evaluated.

4. Work areas. Rooms that have been designated for radiation work are identified by yellow stickers fixed to the doors. As a minimum requirement for work using radionuclides, ensure that you have available: a bench space or tray lined with Benchkote or suitable absorbent material, a plastic bag or beaker for disposal of contaminated plastics, etc., a labeled bottle for liquid waste, a laboratory coat, disposable gloves, and a film badge (if appropriate). When working with $^{32}P$, use a Lucite screen sufficiently long to shield the forehead, and dispose of contaminated tips and solutions into a plastic container behind the screen.

Monitor your work area frequently. Ensure that contaminated material is removed as quickly as possible.

5. Record keeping/waste disposal. All users of radionuclides must keep records. Keep a running log of materials used. Solid waste must be disposed of in an appropriate bin. Scintillation vials containing Optifluor or equivalent high-flash-point solvent must be thoroughly rinsed in a designated sink and then discarded. Make sure that the maximum permissible load to the sewer per day is not exceeded (consult the "Radiation Safety Manual"). Report of sewer disposal by an RC-10 form is required for months in which the quantity of release by the approved user is one control unit or more.

**Peptide Synthesis and the Quantitative Ninhydrin Tests**

Hazardous chemicals

Dichloromethane
Dichlorhexylcarbodiimide/dichloromethane
Dimethylformamide
60% Ethanol/water
Ethanolamine/methanol
Hydroxybenzotriazide/dimethylformamide
Methanol
Ninhydrin/ethanol
Phenol/aqueous ethanol
Potassium cyanide/pyridine
Trifluoroacetic acid (TFA)

Most of these chemicals cause mild to severe irritation when they are ingested, inhaled, or if they touch the skin. For specific hazards and emergency procedures, refer to the Material Safety Data Sheets (MSDS) for each chemical. These sheets are kept on file with the ABI 430A peptide synthesizer in Room 107. Special protective equipment routinely used should include safety glasses with side shields, laboratory coat, and gloves made of butyl rubber or polyvinyl. These chemicals should be handled only in well-ventilated areas and an MSHA- or NIOSH-approved respirator should be used for emergencies and high vapor concentrations. Flammable chemicals should be stored away from heat, sparks, and flame.

General first-aid procedures for these chemicals are as follows. For specifics, consult the MSDS or poison center. Always get medical attention as a routine precaution.

For ingestion, if the person is conscious, give large volumes of water and induce vomiting. Do not induce vomiting if the person is unconscious.

For skin contact, remove contaminated clothing and flush contaminated area with large volumes of water for 15 minutes.

For inhalation, provide fresh air and rest. If breathing is difficult, provide oxygen.

For eye contact, flush eyes immediately with large volumes of water for at least 15 minutes.

**Chemical Waste**

When handling waste, wear protective gloves (butyl rubber or polyvinyl), safety glasses and laboratory coat. Work in a well-ventilated area, preferably in a chemical fume hood. Store waste in glass bottles, which should ideally be kept in heavy cardboard containers inside metal cabinets. All waste must be disposed of according to federal, state, and local regulations. Such disposal is handled by Environmental Health Services, B110 Microbiology, CSU. CSU has disposal request forms, which must be completed in order to have hazardous chemical waste removed. All waste containers must be well marked (responsible person and data collection started) and display their approximate composition. Proper first aid for contamination depends upon the waste components (see attached waste profile).

**Sonication**

The primary biohazard arising from sonication of infectious material (mosquitoes, cell culture, tissues, etc.) is the creation of aerosols. Proper containment and protective clothing must be used at all times. Sonication of infectious material should always be carried out within a vertical flow laminar flow hood, Class II or higher. Good hood practice should be employed. Protective laboratory coats and gloves should also be worn.

**ABI 430A INSTRUMENT WASTE PROFILE**

Emergency phone numbers:
(USA) 415-570-6667 Ext. 999 (UK) 0925-825650

1. Identification.

   The liquid waste from the 430A is collected in a series of four (4) one-gallon bottles located in the rack at the bottom of the instrument behind the front panel doors. This waste is a complex mixture of reagents and solvents, which may have properties of greater hazard than the individual waste components by themselves. Handle this material with extreme caution! Do not dispose of this waste in sinks or drains! This material should be disposed of as a regulated hazardous waste!

2. Approximate Composition. (Standard)[1]

| Material | % | TLV | PEL | CAS# |
|---|---|---|---|---|
| Dichloromethane (DCM) | 56 | 100 ppm | 500 ppm | 75-09-2 |
| Dimethylformamide (DMF) | 32 | 10 ppm | 10 ppm | 68-12-2 |
| Ethanolamine trifluoroacetate | 5 | N/A | N/A | N/A |
| Methanol | 3 | 200 ppm | 200 ppm | 67-56-1 |
| Diisopropylethylamine (DIEA) | 1 | N/A | N/A | 7087-68-5 |
| Dicyclohexylurea | 1 | N/A | N/A | N/A |
| Boc Amino acids | 1 | N/A | N/A | N/A |
| 1-Hydroxybenzotriazole (HOBT) | 1 | N/A | N/A | N/A |
| Phenol, pyridine, ethanol, KCN, ninhydrin | <1 | | | |

3. Physical Data.

   Boiling point, 760 mm: N/A
   Freezing point: N/A

   Specific gravity (H20=1): 1.15 pH range: N/A

   Volatility (vol %): 90% Solubility in $H_20$: Soluble

   Appearance and odor: Colorless liquid with a mild aromatic odor.

4. Fire and Explosion Hazard Data.

   Flash point (Closed Cup), DMF only: 58°C (137°F)

[1]Data were obtained from the synthesis of ACP (65-74) using standard version 1.20 cycles. This sythesis consists of 7 single couplings and 2 double couplings. Percentages of the different waste components will vary, depending on the length of peptide synthesized, the number of single versus double couplings, and the version of the software used.

Flammable limits: (DMF only) 2.2% LEL; 15.2% UEL

Fire extinguising media: Dry chemical, alcohol foam, carbon dioxide, or Halon

Special fire-fighting procedures: Use self-contained breathing apparatus and protective clothing to prevent skin and eye contact.

5. Health Hazard Data.

Exposure limits: See Section III. For DCM, NIOSH has recommended a permissible exposure limit of 75 ppm. The STEL is 500 ppm and the IDLH limit is 5000 ppm. For DMF, the STEL is 20 ppm and IDLH level is 3500 ppm. For methanol, NIOSH recommends a ceiling concentration of 800 ppm. The STEL is 250 ppm and the IDLH level is 25,000 ppm.

Effects of acute overexposure

Swallowing: Harmful if swallowed! Causes severe irritation of eyes, nose, and throat. Higher concentrations may cause liver and kidney damage, loss of consciousness, and death.

Skin: May cause severe irritation or burns. Allergic skin sensitization may also occur.

Inhalation: May cause irritation of eyes, nose, throat, and lungs. Higher concentrations may cause pulmonary edema, unconsciousness, and death.

Eyes: May cause severe irritation or burns.

Emergency and first aid procedures

Swallowing: If the person is conscious, give large quantities of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if the person is unconscious.

Skin: Remove contaminated clothing. Flush the contaminated area with water and wash with mild soap or detergent. Get medical attention.

Inhalation: Provide fresh air and rest. If the person is having difficulty breathing, provide oxygen and get medical assistance immediately.

Eyes: Flush eyes immediately with large amounts of water for at least 15 minutes. Get medical attention.

6. Reactivity Data.

   Stability: Stable

   Incompatibility: Contact with strong oxidizing agents or concentrated acids or bases may cause fire or explosion.

   Hazardous combustion or decomposition products: Burning may release toxic vapors and gases, including phosgene, hydrogen chloride, hydrogen fluoride, carbon monoxide, and oxides of nitrogen.

   Hazardous polymerization: Will not occur.

7. Spill or Leak Procedures.

   Steps to be taken: Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Remove all ignition sources. Small quantities may be collected with absorbent towels or pads and removed to a well-ventilated area away from ignition sources. Larger amounts (1 liter or more) may be collected with an inert absorbent (kitty litter or similar material) or commercially available spill pillows designed for solvent collection. This waste material must not be allowed to enter confined spaces (such as a sewer!) because of the possibility of an explosion.

   Waste disposal: This instrument waste solution should be disposed of as a regulated hazardous waste by a properly permitted hazardous waste management facility in accordance with federal, state and local regulations. Recommended disposal methods include high temperature incineration and solidification for secure chemical landfill disposal.

8. Special Protective Equipment.

   Respiratory protection: An MSHA or NIOSH approved respirator for organic vapors is recommended. A supplied-air or SCBA respirator is recommended for high vapor concentrations and emergency situations.

   Ventilation: Handle within a well-ventilated area. Minimize open exposure to air.

   Protective gloves: Butyl rubber or smaller type resistant to chlorinated solvents.

   Eye protection: Safety glasses with side shields, monogoggles or face shield.

   Other protective equipment: As necessary to prevent skin contact.

9. Special Precautions.

Precautions to be taken: Handle as a flammable, poisonous liquid. Maintain adequate ventilation at all times. Do not breathe vapor. Do not get in eyes, on skin, or on clothing. Accidental contact should be washed away immediately. Keep away from heat, sparks and flame. Keep containers tightly closed. Eye bath, safety shower and spill collection materials should be in area of use.

Other: This waste solution has strong solvent properties and will attack many forms of rubber, plastics, coatings and finishes.

10. Additional Information.

When not directly attached to the instrument, this waste material should be stored in a secure, well ventilated location suitable for flammable materials. Store away from light, heat or potential ignition sources. Contact the appropriate state hazardous waste regulatory agency for proper disposal procedures and lists of the registered service companies.

This waste material is hazardous and should only be handled by persons thoroughly trained in hazardous materials handling procedures!

ACRONYMS USED

ACGIH: American Conference of Governmental Industrial Hygienists.
CAS#: Chemical Abstracts Service compound reference number.
IDLH: Immediately dangerous to life and health.
LEL: Lower explosion limit.
MSHA: Mine Safety and Health Administration.
NIOSH: National Institute for Occupational Safety and Health.
OSHA: Occupational Safety and Health Administration.
PEL: Permissible exposure limit. The federal OSHA limit, usually expressed as a TWA for an 8-hour work shift.
PPM: Parts per million.
SCBA: Self-contained breathing apparatus.
TLV: Threshold limit value. The ACGIH recommended TWA, usually for an 8 hour work shift.
TWA: Time-weighted average.
UEL: Upper explosive limit.

**Trituration of Vertebrate Tissues**

The most common vertebrate tissue to be triturated will be brains harvested from suckling mice. These harvests are generally from mice that have been inoculated with field materials for virus isolation or with virus for stock preparation. Under either of these circumstances, brain tissue is likely to have high concentrations of infectious virus present. Trituration of this tissue is potentially one of the most hazardous laboratory manipulations to the technician if care is not taken to eliminate the creation of and exposure to aerosols.

If animal tissues are unfrozen, they may be placed in a separate pan in a laminar flow biosafety cabinet. If they are frozen, specimens should be placed in a pan and held under the safety cabinet at room temperature for an hour or so before work is to start.

Laboratory coats/smocks and disposable gloves must be worn throughout all phases of work.

Prepare the safety cabinet by placing all necessary items under the hood.

a) Cover the safety cabinet work surface with absorbent plastic-backed paper to absorb any spills.

b) Place in the safety cabinet: a discard pan containing water, necessary pipettes and pipetters, mortars and pestles, sterile sand or alundum, 1% BA in tris-buffered saline diluent, centrifuge tubes, individual petri plates, sand, needles (≥1", 18 ga.) and 3 ml syringes.

c) For tissue other than mouse brain, or if more than a few suckling mice will be harvested, a small balance for weighing tissue will be required in the safety cabinet.

d) Make sure that a spray bottle of 10% bleach solution is handy in case of accidental spills.

Assuming at this point that the tissue being harvested is mouse brain, the following steps should be followed:

a) Using a syringe and needle, insert the needle at the base of the skull and aspirate brain. The brains from a number of mice may be harvested in like manner using the same syringe. Precautions to prevent needle sticks must be observed.

b) Carefully express content of the syringe into a preweighed tube; cover and weigh it.

c) Transfer weighed material to a centrifuge tube.

d) When all harvests have been completed, place the pan with the centrifuge tubes on a cart and move to a refrigerated centrifuge. Centrifuge specimens at approximately 1500 rpm for 20 minutes to sediment the brain tissue. Observe centrifuge precautions.

e) Place all disposable materials into discard pans or plastic biohazard bags for sterilizing in an autoclave. Carefully wipe the balance scale with 70% alcohol before removing from the safety cabinet.

f) Disinfect the work surface of the safety cabinet with the bleach solution, and leave the hood air circulation system on for the balance of the work day.

## ENTOMOLOGY LABORATORIES

### Trituration of Mosquito Pools

Field-collected mosquitoes will have been identified and sorted into pools on a refrigerated surface (chill table), and individual mosquito pools will be placed in glass petri dishes and held in a refrigerator at 4°C until ready for grinding (less than 24 hours).

Prepare the laminar flow biological safety cabinet in which grinding of the mosquito pools will be done.

a) Place plastic-backed absorbent paper on the work surface of the safety cabinet to absorb accidental spills.

b) Add to the safety cabinet a discard pan, chilled grinders, sterile sand, an iced rack of labeled Eppendorf centrifuge tubes, and an iced rack of stoppered tubes that have been filled with diluent.

c) Keep a spray bottle with 10% bleach handy in the safety cabinet to use for decontamination, if necessary.

d) Place disposable gloves ready for use inside the safety cabinet.

e) All laboratory workers will wear an approved laboratory coat or smock at all times when working with known or potentially infected specimens.

Place stacks of petri plates containing mosquito pools in a plastic pan and move them to the safety cabinet where grinding will take place. Using a pan to carry the petri dishes will minimize the chance that the petri plates will be dropped.

Grind pooled mosquitoes in the safety cabinet. In case of a spill or other accident with mosquito suspension, add bleach from a spray bottle to the spill.

When work is completed, clean the safety cabinet and discard used supplies.

a) Remove plastic-backed absorbent paper and place in discard pan.

b) Thoroughly decontaminate the work surface of the safety cabinet with bleach solution and wipe up excess bleach with tissues provided.

c) Place all such paper and gloves in the discard pan, remove the pan, and place it in the autoclave after attaching a piece of autoclave tape.

d) Do not mix mortars and pestles with other discarded materials. Be sure that mortars and pestles are covered with water prior to autoclaving.

**Testing Mosquito Pools for Virus Isolation**

Testing of mosquito pools for virus isolation will be done entirely in a laminar flow biosafety cabinet. Utmost care must be taken to conduct procedures in a manner minimizing creation of aerosols which could result in cross- contamination of specimens. Mosquito suspensions may contain human pathogens, such as St. Louis encephalitis and California encephalitis viruses, for which no vaccines are available. Safe and careful laboratory techniques and the use of safety equipment and supplies, such as a safety cabinet and gloves, are the only way to ensure the safety of workers.

Prepare the safety cabinet in which cell culture inoculation of mosquito suspension will be done.

a) Cover safety cabinet work surface with absorbent plastic-backed paper.

b) Place in the safety cabinet the discard pan containing a 10% bleach solution, pipettors and tips, gloves, disposable Pasteur pipettes and suction flask required to remove cell culture medium.

c) Make sure that a spray bottle containing 10% bleach is available in the event of accidental spills.

d) Follow methods of protocol for virus identification.

Culture plates in this procedure may be overlaid on the laboratory bench top.

a) Place plastic-backed absorbent paper on the bench top.

b) Carry cell culture plates from incubator to bench on a cart to prevent the accidental dropping of trays.

c) Carefully overlay cultures with Cornwall syringe, gently expressing overlay media so that none splashes out of culture plate wells.

d) When plates are overlaid, place in a darkened room while the agar overlay solidifies. Return plates to incubator on a cart.

When work is completed, remove all items from the safety cabinet and discard contaminated supplies.

a) Cover and remove discard pans containing contaminated inoculation tips, pipettes, etc., and place in autoclave.

b) Place paper covering work surface into a biohazard bag, then into a covered discard pan and place in autoclave.

c) Remove equipment, pipettors, and all other supplies from the safety cabinet.

d) Spray work surface of safety cabinet with 10% bleach solution, wipe surface with tissues provided, and place tissues in covered discard pan for autoclaving.

e) Remove gloves and place in covered discard pan to be autoclaved.

Leave safety cabinet exhaust system running throughout the day and turn off just before leaving at the end of the day.

**Intrathoracic Inoculation of Mosquitoes**

Intrathoracic inoculation of mosquitoes will be done in special rooms in the insectary constructed for that purpose. The main features of the rooms are the double door entries and the light-colored surfaces. The main safety concerns during the inoculation procedure are the escape of live, inoculated mosquitoes and accidental needle sticks of workers during the inoculation process.

The following equipment and supplies will be assembled beforehand on the workbench.

Plastic-backed absorbent paper will cover the bench work surface.

Discard pans, disinfectant (10% bleach), glass slides, glass inoculation needles, aspirators for handling mosquitoes, disposable gloves, large glass test tubes in a container of crushed ice, and a special holder for the vial containing the inoculum to be placed on the workbench.

Relatively small numbers of mosquitoes (5-10) will be aspirated from the holding cages in the insectary and carefully blown into glass tubes with cotton stoppers. The tubes will be immediately pushed down into crushed ice to chill the mosquitoes. The immobilized mosquitoes will be brought into the inoculation room in cotton-stoppered test tubes in an ice bath.

The needle is prepared from melting point capillary tubing and carefully calibrated with 1-mm markings. Care must be exercised when drawing the inoculum into the needle so that the volume drawn up does not exceed the capacity of the needle and get drawn into the needle holder. This will contaminate both the needle holder and the virus suspension.

Following approximately 15 minutes of chilling, 2-5 mosquitoes will be spilled from the tube onto a 2" x 4" glass slide on the stage of a stereomicroscope. During handling of mosquitoes, care must be taken to count and keep track of the number of mosquitoes being worked with. As mosquitoes are spilled onto the glass slide, the total number will be recorded on a lab counter.

Care must be exercised when inoculating mosquitoes that the needle does not completely penetrate the mosquito and come out the other side. Expressing the inoculum at this point will result in the creation of an aerosol, and the mosquito will not be inoculated with the appropriate volume of pathogen suspension. Check each mosquito to be sure that the inoculum entered before removing it from the needle.

After inoculation, while the mosquito is still impaled on the needle, place it in a paper holding carton through a small hole in the side which is plugged with cotton or a cork stopper at all other times. The paper-holding carton will be covered on both sides with fine nylon mesh organdy.

When the mosquitoes have been placed into the carton (approximately 15), carefully tape the cork in place to prevent accidental escape of the mosquitoes. Place the cartons in a secure cage before they are removed from the inoculation room.

The number of mosquitoes in each carton, the date, the inoculum (including dilution), the experiment number, and carton number must be be recorded on the carton, and this number checked against the number recorded on the lab counter to be certain that all mosquitoes are accounted for. The same information is recorded in a record book so that all mosquitoes can be accounted for when the experiment is terminated.

When inoculations have been completed, all contaminated materials and supplies should be placed inside a discard pan (needles, glass slides, etc.) or plastic autoclave bag (paper bench liner, gloves) and carried to the autoclave. Autoclave tape should be attached to each pan or bag.

The workbench surface must be sprayed with bleach disinfectant and the surplus wiped up with tissues. The room will be carefully examined for the presence of any escaped mosquitoes. If any mosquitoes escaped and were not recaptured, a warning sign will be placed on both doors of the room, and the supervisor will be notified.

Any accidents such as needle sticks, spilled inoculum, etc., must be promptly reported to the supervisor and the Division Biosafety Officer. When appropriate, as in the case of needle sticks, accident report forms must be completed.

A log book must be maintained inside the inoculation room. The date, time of entry and departure from the room, the pathogen being worked with, the mosquito species, and the names of the operators, must be recorded in the book each time work is done in the room.

**Transovarial Transmission of Arboviruses by Mosquitoes**

Standard Protocol

From 100-150 parent females are used in each experiment. Females will have been in contact with males long enough to have been inseminated but will not have had a blood meal. All subsequent manipulations with live mosquitoes will be done behind two screened enclosures in special rooms in the insectary.

Females are anesthetized by chilling or $CO_2$ and infected by intrathoracic inoculation using the method described by Rosen & Gubler.[1] Infected females are placed in 1-gallon cartons with nylon-mesh tops and tubular stockinet sleeves. Dry oviposition dishes and paper liners are placed in each cage. The stockinet sleeves are twisted tightly, doubled back upon themselves, and secured with two rubber bands. The cages are moved to a locked insectary chamber and held at 27˚C for 5 to 8 days. The mosquitoes are fed during this time through gauze-covered jars containing 5% sucrose, which are placed on top of each cage.

After 5-8 days incubation, mosquitoes are allowed to feed on an uninfected mouse or chick. One or 2 days later the cages are moved to screened Room No. 5, and water is carefully added to oviposition dishes by inserting a water-filled pipet through the stockinet sleeve. A doubled rubber band is kept around the sleeve during this procedure to minimize the possibility of mosquitoes escaping from the cage. After water is added, the sleeves are secured and cages are returned to the locked chamber. The 1-gallon carton cages are transported to and from the locked chambers in a security cage that provides double security against mosquitoes escaping.

Eggs from parent females are collected as a group and hatched in a chamber reserved for infected mosquitoes. Fourth instar larvae may be harvested for viral assay. These should be washed twice in an appropriate diluent, e.g., BA-1, pooled in groups of 50 to 100, triturated in 1 ml of diluent in a tissue grinder and centrifuged using biosafety procedures described above (pp. 68). Supernatants can be frozen in 1-dram vials at -70°C or tested immediately.

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[1]Rosen, L., and D.J. Gubler. Am. J. Trop. Med. Hyg. **23:**1153-1160, 1974.

Eggs are collected on paper strips in plastic cups containing a small amount of water. Following oviposition, cages are moved to screen-room number 5, where oviposition cups are removed with a gloved hand. Excess water in the cups is poured into a common discard container for autoclaving. Plastic tops are snapped onto the oviposition cups, and the cups are returned to the insectary chamber along with the caged adults. Procedures for subsequent gonotropic cycles are the same.

Alternatively, pupae can be collected daily and allowed to emerge in cages. Cages of adult mosquitoes are moved to a screened room and anesthetized for pooling in groups of 100 or less. Males and females are pooled separately. Mosquito pools are placed in stoppered shell vials and frozen at -70°C until processed for viral assay.

Each pool is triturated and centrifuged as described in Item 2 and on page 68. Supernatant fluids are placed in 1-dram screw-cap vials and frozen at -70°C or tested immediately. Viral assays depend on the virus being investigated. Assays in cell culture and mice are performed in the main building. If the FA technique is used, virus suspensions are inoculated into Toxorhynchites mosquitoes and head squashes are prepared (Rosen & Gubler).[1]

Special Precautions

a) All manipulations with live, infected mosquitoes must be carried out in screened rooms in the insectary. Mosquitoes must be in security cages when transported between inoculation chambers and screened rooms.

b) All equipment and materials that are used for inoculation or specimen preparation for viral assays must be autoclaved before discarding.

c) Oviposition dishes and unused eggs and egg strips must be sterilized in an autoclave.

[1]Rosen, L., and D.J. Gubler. Am. J. Trop. Med. Hyg. **23:**1153-1160, 1974.

d) Unused caged mosquitoes should be killed by placing the cage in a plastic bag, securing tightly, and leaving the bag in the freezing compartment of the refrigerator in the insectary for 24 hours.

e) The number of mosquitoes used in each experiment must be recorded. All mosquitoes must subsequently be accounted for. Any discrepancy must be reported immediately to the supervisor.

**Viral Pathogenesis Studies in Mosquitoes**

Following infection, mosquitoes should be maintained in secure holding cages that have been carefully inspected prior to use. Holding cages are then placed in a properly identified, labeled, and locked environmental chamber. Access to the chamber should be limited to those directly involved in the maintenance of the mosquitoes; visitors and the public should not be allowed in areas where exposure to infected arthropods is possible. Holding cages should be properly labeled, including the number/species/strain of arthropods in the cage, the infectious agent used, the experiment number/ID, and the date infected. Holding cages should be inspected frequently during the experiment to ensure their soundness.

When cohorts of infected arthropods are to be sampled during the course of an experiment, the holding cage should be removed in security cages to a secure, screened, working area behind double doors. The working area should be painted white to facilitate the discovery of escaped arthropods and should have all access to ventilation systems covered. Equipment and supplies in the working area should be kept to a minimum to avoid clutter in which escaped arthropods might hide.

Infected mosquitoes should be anesthetized by cold-shock (tropical species) or carbon dioxide gas (cold resistant, temperate species) prior to experimental manipulation (dissection, placement in fixative). If a potentially infected mosquito escapes into the working environment, all work must stop until the released arthropod is recaptured. Entry and exit from the work area must cease during the hunt; appropriate warning signs must be posted if the search becomes protracted.

**Infecting Mosquitoes from Virus Suspensions**

Mosquitoes are exposed to infectious virus by feeding on warm droplets consisting of equal volumes of washed red blood cells and virus. The virus is in the form of freshly harvested infected cell supernatant, suckling mouse brain, or mosquitoes. Mosquitoes (in 1-pint cardboard containers with fine mesh (non-wetable netting) are exposed for 15 minutes. Transfer of mosquitoes from rearing cages into the cardboard containers should be done either in the rearing chamber or in a secure working area as above. Separation of engorged from unfed mosquitoes should not be attempted until all individuals in a container have been anesthetized (as above). Material in contact with virus (cage screening, gauze pads, cardboard containers) should be placed in marked containers/bags and sterilized in an autoclave. Engorged mosquitoes should be counted and placed in secure, labeled, holding cages, as above.

**Infecting Mosquitoes from Infected Vertebrates**

a) Standard Protocol[2]

Preliminary viremia studies on the experimental host animal species should be conducted so that the best periods for infecting the mosquitoes can be predicted with reasonable certainty.

The usual method of infecting an animal is by subcutaneous or intradermal inoculation of approximately 0.02-0.03 ml of low-passage virus suspension diluted to contain 100-1000 suckling mouse intracerebral $LD_{50}$ or equivalent. A blood sample is drawn 1-2 days before inoculation and then once daily after the inoculation for 7-8 days. Precautions to prevent needle sticks should be followed.

To achieve the most avid feeding upon viremic hosts, mosquitoes should be 3-5 days old and deprived of sugar solution for about 12 hours, and of water for 4-8 hours prior to access to the host animal. All transfers into various cages should be done the day before the feeding to avoid using specimens injured through handling. Mosquitoes may be infected in cages of 9 x 9 x 12 inches, about 300 mosquitoes per cage, if the host animal can be immobilized and placed on the cage. Pint-carton cages may also be used for either very small or large animals, with up to 100 mosquitoes per cage. The animal may be placed on the cage or the cage held against the animal, depending upon its size. Infected animals should not be placed inside a mosquito cage as there is danger of being bitten by mosquitoes when removing the animal and a risk of mosquitoes escaping.

Clipping fur or plucking feathers of the host animals should be done before they are inoculated with virus to avoid contamination resulting from tearing of the skin while they are viremic. The means of restraining animals should be humane, tested, and perfected before the actual feeding experiments begin.

Chickens of various sizes and medium- to large-sized birds may be immobilized by binding them in a piece of cloth, with a hole over a plucked area to permit access of the mosquitoes. Rats, guinea pigs, and other rodents will usually crawl tightly into a slender hardware cloth or screen cylinder; the ends can be bent to prevent their moving forward or back. A rabbit can be tied to a restraining board and the mosquito cage applied against a clipped side, or the rabbit may be placed free in a narrow, open-top box and the cage laid against its back. The ears can be taped across the ends of the pint-carton cage and held down with a petri dish lid. Squeeze cages, permitting crowding of an animal into a restricted space to prevent movement, can be used for dangerous animals.

Tranquilizers may be used to advantage, as required. Also, animals can be quieted by intraperitoneal inoculation of sodium pentobarbital. The proper dosage should be determined experimentally prior to the mosquito feeding. An adult guinea pig requires about 0.3 ml of undiluted liquid preparation (50 mg per milliliter of stock). A bird the size of a cardinal requires approximately 0.1-0.3 ml of 1:10 dilution of such stock.

If the feeding period upon the infected animal is 4 hours or more, a blood sample is taken both before the feeding and immediately after. Titration of the two discloses the

[2] From Chamberlain & Sudia, 1967, Methods in Virology, Vol. 1, Academic Press Inc., New York, NY, pp. 63-103.

range of viremia during the feeding. With feeding periods of less than 4 hours duration, the change in viremia is generally negligible and a single blood sample at the close of feeding is usually considered adequate.

Most mosquitoes feed better in the dark, although light may stimulate feeding by some diurnal species. Those hesitant to feed, such as some species of Culex, can be given a half-night or overnight feeding period. The feedings are set up in late afternoon and lights turned out. The cage is inspected about 6 hours later, by using a flashlight covered with red cellophane, to determine the feeding status. If an adequate number have fed, the host animal is removed and a final blood sample is taken; if not, the feeding is continued until morning.

At the close of feeding, the non-feeders and those with noticeably small blood meals are removed and destroyed. The fully engorged mosquitoes are retained for completion of the experiment.

b) Special Precautions

Feeding must be done in screened rooms or mosquito cage, and the host must be placed inside a separate, larger cage.

In cases where it is unsafe and impractical to move the infected host to the insectary, e.g., monkey infected with dengue or yellow fever viruses, feeding may be done in main building by observing the following precautions:

Transfer caged mosquitoes to and from main building in a separate closed container or larger security cage.

Feeding must be done in a room with the door closed.

All potentially contaminated materials, including feeding cages, should be sterilized in an autoclave.

Vertebrate animals should be euthanized with ketamine or $CO_2$ and disposed of appropriately for incineration.

**Tick Biosafety**

In approaching the topic of tick biosafety, the laboratorian should first recognize that there are three levels of risk.

Colonized Ticks

Colony ticks, presumed free of infection, should present no direct health threats; however, they should be securely contained to prevent escape, especially during manipulations such as feeding. Moats and other escape precautions should be used during feeding and when appropriate for other manipulations.

Purposely Infected Colonized Ticks

Colonized ticks that have been infected with B. burgdorferi or other pathogens should be handled with surgical gloves and white gowns that close at the back. When procedures can

potentially produce aerosols (dissection, grinding, squash preps), they must be performed in a laminar flow biosafety cabinet or a microscope safety box. Work surfaces should be covered with white absorbent paper to facilitate visual recovery of ticks.

Slides for dark field microscopy must have a coverslip if they are to be viewed outside a hood. The microscope stage should be wiped down with an appropriate disinfectant when viewing is complete. Slides should be disposed of in a discard pan to be sterilized in an autoclave.

Care should be taken to avoid cuts and puncture wounds. The following accidents must be reported immediately, first to the appropriate supervisor and then to the Biosafety Officer or, in his or her absence, to another medical officer: 1) punctures and cuts; 2) spills and other recognized potential aerosol-producing events; and 3) missing ticks.

Feral Ticks

Feral ticks, depending on species and location of capture, can carry a number of diseases. Among them are Rocky Mountain spotted fever, Colorado tick fever, relapsing fever, tick-borne tularemia, ehrlichiosis, and babesiosis. Rickettsia rickettsii and Francisella tularensis are readily aerosolized, extremely hazardous organisms, which produce fulminant disease and rapid death if infections caused by them are untreated.

Feral ticks must be strictly accounted for. When brought into the insectary they should be logged by species, total number, sex, collection location, and date. Feral ticks must be kept in securely sealed containers on platforms over moats or in other suitable double containment arrangements. They should be kept in locked rooms.

Population counts of feral ticks must be made at a minimum of 2-week intervals. Ticks must be counted before and after laboratory manipulations and all ticks accounted for as live or dead. These figures must be noted on the laboratory log book described below and in the population count log.

A log book must be kept for all manipulations of feral and laboratory-infected ticks. The book must contain the following information: date, laboratorian's name, procedure(s), field collection number, species, collection location, and date. All ticks must be accounted for. Missing ticks must be reported to the laboratory supervisor and Division Biosafety Officer. The laboratory manipulations log book must be kept in a readily accessible location during and after normal duty hours in the event of an unexplained febrile illness in a staff member.

Dissecting, grinding and preparing head squashes of feral ticks must be performed in the laminar flow biosafety cabinet or microscope glove box. Surgical gloves, white gowns that close in the back, and masks must be worn when using the biosafety cabinet or when viewing feral material under the dark field scope. Punctures or cuts, spills, and other possible exposures to aerosol must be reported to the laboratory supervisor and to the Division Biosafety Officer.

The dark field microscope stage should be wiped down with an appropriate disinfectant (alcohol or bleach) after use. Material on slides must have a coverslip before they are removed from the laminar flow biosafety cabinet.

## STANDARD OPERATING PROCEDURES FOR COMMON LABORATORY ACCIDENTS

## LABORATORY SPILLS

A spill of biological material that occurs in the open laboratory may create a serious problem. Every effort should be taken to avoid such occurrences. A spill poses less of a problem if it occurs in a biological safety cabinet provided the outside of the cabinet is not splattered. Directly applying concentrated liquid disinfectant and thoroughly wiping down the internal surfaces of the cabinet will usually be effective in decontaminating the work zone, but gaseous sterilants will be required to disinfect the interior sections of the cabinet. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

a. Spill in a Biological Safety Cabinet

A spill that is confined to the interior of the biological safety cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity, which will help clean the surfaces by removing both dirt and microorganisms. A suitable disinfectant is a 3% solution of an iodophor such as Wescodyne or a 1:100 dilution of a household bleach (e.g., Clorox) with 0.7% nonionic detergent. The operator should wear gloves during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. This disinfectant, gloves, wiping cloth and sponges should be discarded into an autoclave pan and sterlized in an autoclave. This procedure will not disinfect the filters, blower, air ducts or other interior parts of the cabinet. The entire interior of the cabinet can be sterlized by the formaldehyde gas method, using powdered or flake paraformaldehyde. Calculate the volume of the cabinet in cubic feet and weigh out 0.3 g of flake paraformaldehyde for each cubic foot of space. Place the paraformaldehyde in the frying pan and place the pan in the cabinet with the electric line run to the outside of the cabinet. Raise the humidity within the cabinet to about 70%. Vaporizing water in the frying pan is a convenient technique. Set the thermostat of the frying pan containing the paraformaldehyde at 450° F. Seal the cabinet opening with sheet plastic and tape. If the cabinet exhaust air is discharged into the room, attach flex hose to the cabinet exhaust port and extend the hose to the room exhaust grill; however, if the building exhaust air recirculates, attach flex hose to an open window or door. If the cabinet exhaust is vented directly into the building system, close the exhaust damper. Plug in the frying pan to depolymerize the paraformaldehyde. After one-half volume of paraformaldehyde has been depolymerized, turn on the cabinet fan for about 3 seconds to allow the formaldehyde gas to reach all areas. After depolymerization is complete, again turn on the cabinet fan for 3 seconds. Then allow the cabinet to stand for a minimum of 1 hour. After the 1-hour exposure, open the flex hose on the exhaust damper, slit the plastic covering the opening and turn on the cabinet fan. Ventilate the cabinet for several hours to remove all traces of formaldehyde.

b. Spill in the Open Laboratory

If potentially hazardous biological material is spilled in the laboratory, the first essential is to

avoid inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and go directly to a wash or change room area. If clothing is known or suspected to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated areas as well as the arms, face and hands. Shower if facilities are available. Reentry into the laboratory should be delayed for a period of 30 minutes to allow reduction of the aerosol generated by the spill. Advance preparation for management of a spill is essential. A "spill kit," including leakproof containers, forceps, paper towels, sponges, disinfectant, respirators, and rubber gloves, should be readily available. A high-intensity, portable ultraviolet lamp is useful in emergency situations. This UV lamp can be moved into the room where the accident occurred and the automatic timer set for a given period of exposure. A delay timer allows sufficient time to get out of the room before the UV lamp is automatically activated. The door to the room should be locked or a sign posted on the door warning personnel not to enter as 1200 watts of radiation is emitted by this lamp. A 2-3 hour exposure will sterilize microorganisms that either may be airborne or have settled on exposed surfaces. Radiant energy at 253.7μ has little penetrating power so that microorganisms covered with dirt or dust will probably not be affected.

Protective clothing should be worn when entering the laboratory to clean the spill area: rubber gloves, autoclavable footwear, an outer garment, and a respirator. If the spill was on the floor, do not use a surgical gown that may trail on the floor. Take the "spill kit" into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. Using a hypochlorite solution containing 1,000 ppm of available chlorine, an iodophor solution containing 1,600 ppm of iodine, or other appropriate disinfectant, carefully pour the disinfectant around and into the visible spill. Avoid splashing. Allow 15 minutes contact time. Use paper cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a discard container at they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an autoclave and sterilize. Remove shoes, outer clothing, respirator, and gloves and sterilize them in an autoclave by exposing them to ethylene oxide. Wash hands, arms, and face or, if available, take a shower. If gaseous disinfection of the laboratory room is to be carried out, follow the procedures as outlined by the procedure employed. Gaseous disinfections are performed under supervision of the Biosafety Officer.

**Neutralization and Cleanup of Acid Spills**

Neutrasorb Acid Neutralizer can be used to neutralize the following acids:

1. Hydrobromic acid
2. Hydriodic acid
3. Hydrochloric acid
4. Nitric acid
5. Perchloric acid
6. Phosphoric acid
7. Sulfuric acid

Caution:

1. Neutrasorb should not be used on hydrofluoric acid, peroxy (per-) organic or other highly reactive acids not listed above.

2. Wear adequate protective clothing (safety glasses, laboratory coat, and gloves).

3. Wear an appropriate full-face respirator (with acid gas canister) if the acid spill volume is greater than 1-pint or if adequate ventilation cannot be maintained.

Directions:

1. Apply the Neutrasorb to the acid spill from its perimeter inward. Cover the entire spill surface.

2. After foaming has subsided, add water in small amounts, and mix with scoop. Mix until all foaming ceases and the slurry maintains a blue color throughout. (See color code below.)

Note: If a blue color is not obtained in Step 2, add additional Neutrasorb Acid Neutralizer and water, and continue mixing until the slurry is blue throughout.

Color code: Red/Pink - Highly acidic
Yellow/Buff - Slightly acidic
Blue - Safe

3. Pick up the neutralized material with a scoop and put it in a plastic bag. The plastic bag should be labeled as containing Neutrasorb Acid Neutralizer and alkali metal and alkaline earth salts of the treated acid.

4. Placed sealed plastic bag in the waste chemical storage room.

Caution:

Neutrasorb Acid Neutralizer may cause irritation. Avoid contact with eyes, skin, and clothing. Avoid breathing its dust. Wash thoroughly after handling.

**Cleanup of Solvent Spills**

Solusorb Solvent Absorbent can be used to absorb common organic liquids.

Important:

Immediately remove all sources of ignition from spill area.

Immediately provide maximum ventilation.

Avoid excessive inhalation of vapors or contact of skin with solvents.

Do not use for the cleanup of strong oxidizers, including peroxides, or other highly unstable organic compounds.

Caution:

1. Wear adequate protective clothing (gloves, safety glasses, and laboratory coat).

2. Wear an appropriate full-face respirator (with organic vapor canister) if adequate

ventilation cannot be maintained.

Directions:

1. Apply the Solusorb absorbent to the spill from its perimeter inward. Thoroughly cover all spilled solvent.

2. Using a scoop, thoroughly mix the Solusorb Solvent Absorbent and solvent, until it regains its appearance as a dry, free-running, nonadhering granular material.

3. Using a scoop, pick up the saturated Solusorb Solvent Absorbent and place it in a plastic bag. Seal the bag tightly and label.

4. Place the sealed plastic bag in the waste chemical storage room.

Caution: Do not store used Solusorb Solvent Absorbent at temperatures in excess of 125 F.

**General Safety Information**

Some caustic solutions, notably ammonium hydroxide, produce irritating fumes. For spills involving these materials, ensure adequate ventilation before beginning neutralization and cleanup. For spills of ammonium hydroxide of 30-70% concentration or greater, it is recommended that a respirator or self-contained breathing apparatus be worn. (GMR-C cartridge on full-faced respirator)

Neutralization of concentrated caustics produces heat. Observe appropriate precautions when handling hot materials. Do not step into spill material.

Procedure

1. Put on safety glasses, gloves, and a laboratory coat.

2. Apply Neutracit-2 to the liquid caustic spill from its perimeter inward to dike and absorb the spill. A color change from yellow to blue signifies caustic material (hazardous).

Note: This step will be accompanied by slurry boiling if concentrated caustic solutions are treated. Observe appropriate precautions when handling hot materials.

3. Thoroughly mix the slurry with a scoop until it changes to a yellow/yellowgreen color (neutralized). Add additional Neutracit-2 and/or water, if necessary, to obtain the above color reaction.

Note: This step will be accompanied by slurry boiling if concentrated caustic solutions are treated. Observe appropriate precautions when handling hot materials.

4. Pick up the neutralized material using a scoop, and place in a plastic bag. Label the container as follows:

   "Contents are citrate salts of _____.
   (name of caustic)

5. Place the sealed plastic bag in the waste chemical storage room.

Mercury Spill

All spills of mercury should be cleaned up immediately. Vacuum cleaning is an effective method for removal of mercury. However, vacuum cleaners should be equipped with charcoal filters so that mercury vapor will not be discharged into the work room air. Sweeping should be avoided because it creates dust and tends to break up any elemental mercury into even smaller particles, thereby increasing the rate of vaporization.

Mercury vapor depressants, such as calcium polysulfide, have proved successful in controlling production of mercury vapors from spills. Another product used is the J. T. Baker Mercury Spill Cleanup Kit. See attached directions for specific instructions. Use the

attached label (CDC 0.886) to identify waste and contaminated clean up materials and place in waste chemical storage room. Call the Office of Biosafety (ext. 3883) if further assistance or information is needed.

Mercury Spill Cleanup Directions

The Hazards:

The toxicity of mercury is such that the element and its compounds should not be allowed to contaminate air or water.

General Safety Information:

a) Clear the general spill area before beginning cleanup.

b) Avoid any unnecessary contamination of clothing or equipment.

c) Remove any exposed gold, copper, or silver jewelry.

d) Wear protective gloves and a laboratory coat or apron.

e) Maintain adequate ventilation.

Procedure

1. Using wooden spatulas, gather as much mercury together as possible. Retrieve this material using a mercury aspirator.

Mercury Aspirator Directions

Retrieval:

a) Squeeze the bulb to exhaust air from the aspirator.

b) Place the nozzle tip next to mercury drops and release the bulb sharply.

c) Repeat until the bulb feels about half full.

Caution: Never vent air from the aspirator with the nozzle pointing upward!

Emptying:

a) Invert the aspirator (nozzle up) and remove the tube and collar assembly.

b) Empty the aspirator into a narrow-mouth bottle labeled "Impure Mercury."

c) Reinsert the tube and collar assembly into the aspirator.

Retrieve as much mercury as possible using the above method, then proceed to step 2.

2. Activate the Cinnasorb™ Elemental Mercury Absorbent Base (Prod. No. 4505) using the Cinnasorb Elemental Mercury Absorbent Activator. See instructions

and cautions on individual product labels.

Cinnasorb Base Activation:

a) Prepare Cinnasorb Activator by adding water to the "fill" line on the label. Shake until the crystals are dissolved.

b) Fill the mixing cup supplied about 1/3 full with Cinnasorb Base. Mixing with wooden spatula, add sufficient Activator solution to form a soft paste. Note: Some heat and gas may be formed during activation.

3. Apply a narrow strip of activated Cinnasorb Base to the spill surface and push across the contaminated area using a wooden spatula. Small droplets of mercury will be absorbed into the paste. Repeat until the entire area has been covered at least twice. Activate additional Cinnasorb Base as necessary for complete treatment.

4. Using the plastic scoop provided, scrape up the Cinnasorb absorbent waste and transfer to the wide-mouthed jar supplied. Do not close the jar. Complete and affix one mercury waste material label to the jar. Set it aside and allow the contents to dry thoroughly before closing.

5. Wipe up the treated spill area with a sponge moistened with warm soapy water.

6. Place contaminated sponge, spatulas, gloves, etc., in the plastic disposal bag provided and seal with tape. Complete and affix a second mercury waste material label. Dispose of waste materials (Step 4 and Step 6) in accordance with local environmental regulations.

7. For persistent mercury vapor treatment, use Resisorb™ Mercury Vapor Absorbent to absorb mercury vapors given off by small amounts of mercury remaining in areas inaccessible to physical cleanup.

Sprinkle Resisorb into any cracks or areas where the presence of mercury droplets is suspected. Check mercury vapor levels periodically using a UV absorbance type vapor detector, and renew the Resisorb absorbent as necessary.

**Radiation Safety**

Action Levels for Decontamination Beta and Gamma Emitters

| Smear Results* | Action |
|---|---|
| 100 dpm/100 sq cm | No action required by Radiation Safety Officer (RSO). Left to discretion of authorized user. |
| 100-350 dpm/100 sq cm | Area or surfaces should be cleaned as soon as possible by the authorized user or laboratory personnel. Shoe covers |

| | |
|---|---|
| | and stepoff pads should be used if contamination is on floor. |
| 350-2,000 dpm/100 sq cm | Contaminated area should be cleaned immediately, under supervision of Biosafety Officer (BSO). Shoe covers and step-off pads are required for entry into area. Only essential personnel will have access. |
| 2,000 dpm/100 sq cm | Air flow should be shut off. Entry of personnel into area should be prevented until a representative of Biosafety Officer (BSO) arrives. Cleanup should begin immediately by authorized user under supervision of Biosafety Officer (BSO). Shoe covers and step-off pads are required. |

*dpm = disintegrations per minute
sq cm = square centimeters

Cleanup must be undertaken by authorized users or laboratory personnel, not by custodial workers.

Waste Disposal

No radioactive material may be disposed of without the knowledge and consent of the Biosafety Officer (BSO) and the Radiation Safety Officer (RSO). Methods for disposal must be approved before any actual disposal. No radioactive waste will be accepted for disposal by the Biosafety Officer unless it is labeled with the name of the isotope, the activity date of assay, and the date of disposal.

Warehouse supply will supply authorized users with a standardized radioactive waste container for each isotope used in the laboratory. Each container will be used for ONE isotope only. A separate can will be issued for materials contaminated with a mixture of isotopes, and disposal procedures will be based on the longest half-life. The CDC warehouse will supply plastic bags for lining the waste containers. The radioactive waste cans should be stored in an area within the laboratory where they will not be knocked over, used for other waste, or accidentally mistaken as cans for nonradioactive waste. The area where the waste is stored must be marked with a "Radioactive Waste/Do Not Remove" sign. Authorized users are responsible for securing waste until the Disposal Officer removes it. They can arrange for waste to be picked up by contacting the Radiaton Safety Officer (ext. 3883). Scheduling should be done in advance to prevent waste overflow. Waste is housed in the radioactive waste storage area adjacent to Building 5 until it is permanently disposed of. Except for Biosafety Office personnel, access to this area is prohibited.

All individual bags and bottles of radioactive waste must be marked with a label, form CDC 0.999, Radioactive Waste for Disposal (Appendix H), which provides a place to include all isotopes, their quantities, the date assayed, and their physical form. Any chemical information that might be useful should also be included (for example, strong acid). These individual

disposal containers must then be placed in the large waste can provided for the particular isotope. Inventory sheets, supplied by the Radiation Safety Officer, should contain a complete list of the isotopes disposed of in the waste cans.

No radioactive waste will be accepted for disposal if nonradioactive waste or trash is mixed with it. Each bag must be properly labeled, and the inventory form on the top of the container must be complete.

Containers bearing a radioactive label, but that no longer contain radioactive material, must be disposed of as ordinary trash but only after the radioactive label is defaced or removed.

Solid Waste--Solid waste includes test tubes, beakers, absorbent paper, gloves, pipettes, and other dry items contaminated with radioactive material. This material must be placed in plastic bags, sealed with tape. Hypodermic needles, capillary pipettes, and other sharp objects must be placed in puncture-proof containers before being put into the large waste cans.

Before any radioactive material contaminated with a biological organism (virus, fungus, or bacteria) is disposed of, it must be heat sterilized or chemically treated in a manner that destroys all living organisms. Care should be taken to protect autoclaves from any radioactive contamination.

Before animal experiments with radioisotopes are begun, the Radiation Safety Officer must be consulted so that proper arrangements can be made for disposal of radiologically contaminated or infectious carcasses. Animals that contain less than 0.05 microcurie of hydrogen-3 or carbon-14 per gram can be disposed of as biological waste. At concentrations higher than this or for other isotopes, the animal or tissues must be disposed of as radioactive waste (see Section II R, Use of Radioactive Materials in Animals).

Organic Liquid Waste--Scintillation vials that contain less than 0.05 microcurie of hydrogen-3 or carbon-14 per gram of scintillation medium should be disposed of as chemical waste and not as radioactive waste. All scintillation vials containing radioactivity above these levels must be labeled as radioactive waste. Scintillation fluid and radioactive waste must be left in the original vials for disposal. These vials should be placed upright in shipping trays rather than in the large waste cans.

Solvents that are insoluble, flammable, or toxic must be collected in inert, airtight plastic bottles and must never be disposed of in the sink.

Aqueous Liquid Waste--No liquid radioactive waste shall be disposed of by the sewage system unless (1) the liquid is readily soluble or dispersible in water, and (2) the material is diluted to the concentrations shown in Table A or flushed simultaneously with measured amounts of water sufficient to achieve those concentrations (for example, wash water from glassware that has been used for processing radioactive materials could be disposed of through the sewer).

Table A*
Concentrations of Radiation Above Natural Background, by Isotope

| Isotope | Concentrations (microcuries/ml) |
|---|---|
| $Carbon^{14}$ | $8 \times 10^{-4}$ |
| $Hydrogen^{3}$ | $3 \times 10^{3}$ |
| $Iodine^{125}$ | $2 \times 10^{7}$ |
| $Iodine^{131}$ | $3 \times 10^{-7}$ |
| $Nickel^{63}$ | $3 \times 10^{5}$ |
| $Phosphorus^{32}$ | $2 \times 10^{-5}$ |
| $Sulfur^{35}$ | $6 \times 10^{-5}$ |

*10 CFR 20, Appendix B, Table II, col. 2

Only one sink in each laboratory shall be used for disposing liquid radioactive waste, and it shall be appropriately labeled. After each disposal, the sink shall be flushed with a large amount of water. Authorized users shall keep a record of quantities and isotopes disposed of in this manner and include such disposals on their inventory reports. Chemicals normally treated as hazardous waste cannot be disposed of in this manner.

Liquid radioactive waste must be stored in unbreakable, airtight bottles or in double containers with enough absorbent material in the outer container to absorb any spillage. RIA kits containing I-125 should be treated as radioactive waste and will be disposed of by the Radiation Safety Officer.

Cobalt-60 and Cesium-137 Irradiators

Only persons listed on the NRC license as users shall operate the gamma-cell irradiators without personal supervision. All others must have one of the listed authorized users present. The room housing the irradiators must be locked at all times, and a TLD radiation exposure badge must be worn when the gamma-cell is used. Each user must also sign and date a logbook before using the irradiators. All notices from NRC concerning the gamma-cell irradiators must be provided to each user.

Each gamma-cell will be tested for contamination and leakage at least once every 6 months by a contract vendor. This test must be capable of detecting 0.05 microcurie of contamination. If 0.05 microcurie of removable contamination is found, the gamma cell will be removed from operation immediately.

Each chromatograph will be tested for leakage every 6 months by the laboratorians, with the assistance of the Radiation Safety Officer. Alpha emitters will be tested every 3 months by the authorized users, and records of testing must be sent to the Radiation Safety Officer. Test wipes must be made from the surface of the device where the foil is mounted, according to instructions enclosed with the chromatographs. The radiation detector and the method used for testing must be capable of detecting 0.005 microcurie of contamination. If 0.005 microcurie or more of contamination is detected, the unit must be immediately withdrawn from use.

Gas Chromatography Detectors

All gas chromatography units containing radioactive material must be used under the direction of the Radiation Safety Officer. These sources are subject to the same regulations as other radioactive material at CDC, and the following requirements must be satisfied for each unit:

1. Each piece of equipment must be marked with a radioactive label identifying the isotope, quantity, and assay date.

2. Radioactive foils must not be removed from their cells or transferred to another chromatograph.

3. Chromatographs must be done on absorbent paper, in a hood. Gloves must be worn during cleaning operations.

4. Units with titanium or scandium tritide foil must be used with a properly operating temperature control mechanism to prevent the foil temperature from exceeding 225ºC and 325ºC, respectively.

5. All new equipment or newly repaired chromatographs must be leak tested by the laboratorian before being operated.

6. Gas chromatography units with radioactive foils must be vented into a fume hood or room exhaust vent with plastic tubing to prevent work areas from being contaminated.

7. Units must be operated according to manufacturer's instructions.

8. Authorized users of gas chromatographs with radioactive material are responsible for the security of the source. The equipment or source must not be moved to another location or transferred to another user without permission from the Radiation Safety Officer.

9. The Radiation Safety Officer must be informed of any radioactive foil that is no longer in use.

Each chromatograph will be tested for leakage every 6 months by the laboratorians, with the assistance of the Biosafety Officer. Alpha emitters will be tested every 3 months by the authorized users, and records of testing must be sent to the Radiation Safety Officer. Test wipes must be made from the surface of the device where the foil is mounted, according to instructions enclosed with the chromatographs. The radiation detector and the method used for testing must be capable of detecting 0.005 microcurie of contamination. If 0.005 microcurie or more of contamination is detected, the unit must be immediately withdrawn from use.

X-ray Equipment

Only authorized users will be permitted to use x-ray-producing equipment. These authorized users must meet the same general requirements and will have the same responsibilities as other authorized users of radioactive materials. All applicable sections of the Radiation Safety Manual must be followed. The following is a partial list of requirements:

1. Process all orders for x-ray equipment through the Radiation Safety Officer.

2. Complete an authorized user form, and wait for the request to be approved before using the equipment.

3. Obtain Radiation Safety Officer approval for the location of the machine and the operating procedures before using it.

4. If the x-ray beam is not fixed in position, check and record its alignment quarterly. Realign, if necessary.

5. Enforce security precautions stringent enough to prevent any non-authorized personnel from using the equipment at any time.

6. Test safety interlocks every 6 months to see that they are functional.
7. Follow guidelines provided by the Radiation Safety Officer for the specific conditions under which the x-ray equipment is used. Authorized users must meet the requirements established by the Biosafety Officer.

Radioactive Phosphorus

Phosphorus-32 emits high-energy beta radiation. Shielding for this type of radiation should be composed of a material with a low atomic number, such as plastic or plexiglass. Phosphorus must be stored and disposed of in plastic containers and must be kept cool at all times.

Use of Radioactive Materials in Animals

Before animal experiments with radioisotopes are begun, the Radiation Safety Officer must be consulted so that proper arrangements can be made for disposal of radiologically contaminated or infectious carcasses.

Radioisotopes must be administered to animals over an absorbent material that can be easily removed if contaminated. Cages housing these animals must be labeled with the isotope, the quantity injected per animal, and necessary precautions (if isotope is eliminated in feces, care should be taken with waste material). These cages should be segregated from other cages, if possible. Animal excreta may be disposed of by sewer if the concentrations do not exceed those shown in Table 5. If concentrations are likely to be above this level, the waste should be bagged and handled as solid radioactive waste. Animal carcasses containing less than 0.05 microcurie of hydrogen-3 or carbon-14 per gram can be disposed of as biological waste. Carcasses with higher concentrations or containing other isotopes must be disposed of as radioactive waste. Carcasses or tissues should be frozen in a plastic bag and labeled with a radioactive decal that includes species, isotope, and quantity, and they should remain frozen until permanent disposal is available.

All workers handling the animals must be informed of necessary procedures and safety measures. If the radioactive material is capable of becoming volatile after the animal is infected, for example, through exhaled air, proper ventilation must be provided to ensure that radioactive material does not accumulate in room air.

Emergency Procedures

Advise the Radiation Safety Officer of emergency situations.

General Procedures

All users of radioactive materials should be thoroughly familiar with these procedures before any emergency arises. When an accident involving radiation occurs, address the greatest hazard first; lifesaving measures always take precedence over decontamination or other concerns. Advise personnel working nearby of any hazard or accident as soon as possible, and prevent them from entering the hazardous area. Always notify the Radiation Safety Officer (RSO) and Biosafety Officer (BSO) (ext. 6422) when an accident occurs.

Office of Biosafety

The Biosafety Officer will investigate all accidents, spills, fires, or other incidents in which radiological material is involved. In the event of an accident, the Biosafety Officer will assist by monitoring personnel and cleanup procedures and by providing technical advice.

The Biosafety Officer, through the Radiation Safety Officer (RSO) and the Radiation Safety Committee (RSC), has the responsibility of planning and arranging emergency medical care for victims contaminated with radioactive material or overexposed to radiation at CDC facilities. The Biosafety Officer will ensure that procedures for emergency care and a list of phone numbers and contacts are made available to all authorized users.

Specific Procedures

1. Explosion

    a. If an explosion occurs, assume that the entire laboratory is contaminated.

    b. Perform any possible lifesaving measures that are needed.

    c. Call the Biosafety Officer immediately, or have someone call for you. Inform the Biosafety Officer of any injuries, particularly life-threatening conditions.

    d. Turn off all fume hoods and ventilation where possible.

    e. If possible, evacuate the area of the explosion. Restrict contamination to the area by removing your gloves, shoes, and laboratory coats before leaving.

    f. Wash your hands and arms thoroughly with a mild soap for several minutes, and rinse them thoroughly with cool water.

    g. Flush superficial wounds with water and cover them with clean, sterile material. Seek medical attention at the Poudre Valley Hospital Emergency Room.

    h. Do not attempt to clean up without the supervision of the Radiation Safety Officer.

    i. Do not continue work in the laboratory without the Radiation Safety Officer's approval.

2. Fire

   a. Call the fire department (dial 911) from a safe place, and identify the location of the fire.

   b. Call the Biosafety Officer.

   c. Try to extinguish the fire without risking the safety of personnel.

   d. Avoid spreading the contamination.

   e. Do not continue work in the laboratory without the Biosafety Officer's approval.

3. Spills

   a. Inform the occupants of the laboratory about the spill.

   b. Cover the spill with absorbent material as quickly and as completely as possible to prevent spreading. To localize the contamination, wipe inward toward the center of the spill. Do not wipe back and forth or in a random fashion.

   c. Have someone who is not contaminated call the Biosafety Officer immediately.

   d. Remove shoes, gloves, and laboratory coat before leaving the laboratory.

   e. Wait for a representative of the Biosafety Officer before attempting any cleanup.

   f. If at all possible, do not touch objects or people before being checked for contamination.

   g. If a biological agent is involved, soak the area with a disinfectant for 30 minutes to inactivate the agent, and wash hands and arms thoroughly with soap or an appropriate disinfectant. Scrub hands for several minutes and rinse them thoroughly.

   h. Anytime you leave the contaminated area subsequent to clean up, remove your gloves, shoes, and laboratory coat; segregate them as radioactive waste before leaving the laboratory.

   i. If toxic or radioactive fumes are generated outside the hood, turn on all fume hoods.

   j. Do not continue using the laboratory without the approval of the Radiation Safety Officer and the Biosafety Officer.

4. Accidents Involving Large Sources

   a. If there is any reason to suspect that a large source such as the 4 gamma-cell is unshielded or leaking in any way, immediately evacuate all personnel to a safe area.

   b. Call the Radiation Safety Officer.

   c. Have any person who may have been exposed remain in a safe area until a Biosafety Officer representative arrives.

## Bibliography

International Commission on Radiological Protection. The principles and general procedures for handling emergency and accidental exposure to workers; publication 28. Elmsford, New York: Pergamon Press, 1978.

U.S. Nuclear Regulatory Commission, Washington, D.C., Nuclear Regulatory Guides:

8.10 Operating philosophy for maintaining occupational exposures as low as reasonably achievable, 1975.

8.13 Instruction concerning prenatal radiation exposure, 1975.

8.20 Applications of bioassay for I-125 and 131, 1979.

8.29 Instruction concerning risks from occupational radiation exposure, 1981.

Shapiro, J. Radiation protection. 2nd ed. Cambridge, Massachusetts: Harvard Univ. Press, 1981.

## PUNCTURE WOUNDS

### Animal Bites - General

All animal bites, injuries, exposures to hazardous agents, or unusual illnesses are reported by the following procedures.

a) Report to Biosafety Officer.

b) Complete CDC Accident Report (Form 0.304) to receive authorization for medical treatment.

c) Complete Form CA-1 to report an on-the-job injury or complete Form CA-2 to report a disease condition as a result of an on-the-job exposure to hazardous agents.

Form C-16 is completed when a private medical facility is authorized for work-related traumatic injury.

All bites shall be immediately washed thoroughly with hand soap (Septisol) and warm water and flushed for at least 3 minutes with water. Tincture of iodine is then applied and the individual reports to a medical officer.

The biting animal is examined and observed for disease as indicated.

Working with Primates

Personnel entering a room or enclosure housing primates must:

Wear a mask or face shield.

Wear protective clothing over street clothing or change into protective clothing (coveralls, boots, gloves, etc.).

Use devices that protect from bites such as leather gloves and nets when catching primates. Chemical restraint (Ketamine at a dose of 5-15 mg/kg I.M.) should be used where possible, and especially when handling aggressive monkeys.

Remove protective clothing before leaving the facility.

Have yearly tuberculin skin tests and/or chest radiographs.

### Macaque Bites

Following a bite or scratch from a macaque that breaks the skin and causes bleeding:

Immediately and thoroughly cleanse the site by using liquid soap (Septisol), water, and a surgical scrub brush.

Soak the wound site for 15-20 minutes using gauze sponges that have been

immersed-in Dakin's solution.[1] Change the sponges every 5 minutes while soaking.

Using sterile swabs, obtain a culture from the conjunctiva and tonsillar area of the monkey into 3 ml of veal infusion broth containing 1% penicillin and streptomycin.

Draw blood from the monkey and prepare a 2-ml acute-phase serum sample for storage.

Arrange for emergency medical care follow-up of patient (examination by physician, suture of wound if indicated, tetanus shot, blood drawn for serum to be sent as a follow-up to the laboratory for acute-phase, then convalescent-phase titer.

Report to the Biosafety Officer.

**Contaminated Needles or Other Sharp Objects**

Treatment

Percutaneous injuries are vigorously scrubbed and soaked for 5 minutes with povidone-iodine or 3% solution of hydrogen peroxide or washed with soap and water; contaminated mucous membranes are irrigated or rinsed for 15 minutes with normal saline (an eyewash kit is useful for this purpose).

If the history of the incident indicates, or the laboratory worker requests it, a focused physical examination is performed by a physician for each person reporting an overt exposure. If the laboratory worker or supervisor has not completed a laboratory incident form for the Biosafety Office (BSO), the physician is obligated to advise the Division Director, NCID Director, and the Biosafety Officer of such incidents and will have to identify the laboratory(s) where the incident occurred without disclosing the individuals involved by name beyond the Division Director.

Serologic Monitoring/Immunologic Profile

Specimen collection and processing will be performed; 30 ml of blood is also drawn to test the serum and to separate lymphocytes for storage for possible future virus isolation.

Work Site Investigation

Once laboratory exposure is reported, a prompt initial investigation of the involved laboratory will be conducted by the Biosafety Officer. If the involved worker(s) shows seroconversion at any test interval and the physician indicates a possible laboratory exposure, the Biosafety Officer, upon request, will again conduct a complete investigation of laboratory work practices; the Biosafety Officer will make every effort to protect the identity of the laboratory(s) involved throughout the investigation. The report of the Biosafety Officer is submitted to the Director of the Division of Vector-Borne Infectious Diseases.

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[1]Dakin's solution is diluted, buffered sodium hypochlorite, which has a shelf life of 1 month. If Dakin's solution is unavailable or outdated, use a mixture of 9 parts water to 1 part household bleach; this solution has a shelf life of 24 hours.

Follow-Up

The participant is advised to report and seek medical evaluation of any acute febrile illness, rash, or swollen glands that occur within 3 months of an overt exposure; blood collection is performed at 3 to 6 weeks, 3 months, and 6 months following the report of an overt exposure or injury; if the results are negative after the 6-month specimen collection is tested, the individual then returns to the 4-month interval schedule.

## BIOSAFETY RULES PECULIAR TO THE SAN JUAN LABORATORIES (SJL)

Building Design and Security

1. All employees and authorized visitors use a card to gain entry to the premises through the electronic gate.

2. All other persons requesting entry to the premises must communicate via speaker. Electronic gate is opened only when the request is judged legitimate and important for the functions of the laboratory.

3. Keys to the building (and insectary) are issued only to the laboratory employees, contract service personnel, and authorized visitors.

4. Unauthorized persons are not permitted to enter in the laboratory wing beyond a sign next to the restroom. No children are permitted to enter in the laboratory wing beyond the point above.

5. Employee identification cards have been issued to SJL CDC staff, and should be worn in work areas.

General Practices

1. All employees and visitors who work in the laboratory wing, manipulate clinical specimens or infectious microorganisms, will receive a copy of the biosafety manual from the Biosafety Officer and basic instructions are provided during the first week after their appointment or arrival at SJL.

2. Protective gloves must be worn at all times when handling clinical specimens or viruses in a laminar hood. Air flow in the hood is stopped only when absolutely necessary (e.g., when centrifuge tubes are balanced).

3. Sharps are deposited in a designated waste disposal container with detergent solution (Isolyser$^{R}$). When the container is 3/4 full, catalysts are added, the wastes are solidified, and are disposed of as noninfectious waste materials.

Obtaining Serum Specimens from Outpatients

Blood is drawn from outpatients by a medical technologist or a physician in a designated room provided with a bleeding chair. Protective gloves must be worn by the phlebotomist.

Field Assignments

Employees who enter private premises as part of their work assignment must wear a photo-identification badge issued by SJL.

Vaccination and Health Insurance for Visitors

1. All potential visitors to the SJL are provided with a note requiring hepatitis B and YF vaccinations prior to their arrival in San Juan and the procedure for obtaining insurance coverage for the stay.

Fire Safety

In case of fire, two fire exits in the office wing and two designated fire exits and a door to the stockroom are used in the laboratory wing. In the insectary, a front door and a rear door (to be installed) are used for escape.

**Disaster Emergency Plan**

The San Juan Laboratories (SJL) Emergency Response Plan describes the actions to be taken during natural emergencies and provides a framework for SJL's response to such events.

This response plan is not all-inclusive. All response activities cannot be included here, and unexpected actions will inevitably be necessary. The plan should ensure that when an emergency requires action by CDC, the resulting response will be immediate, effective, and coordinated.

Another objective of this plan is to provide technical information, health advice and other assistance to state and local officials for the re-establishment of public health order.

Response to a Natural Disaster

In case of a flood, hurricane, earthquake, storm, or other catastrophe, the following actions will be taken:

Before the disaster (If warning is available): The Director of SJL will instruct employees to:

1. Inspect, start, and run the power generators for 5 minutes. The diesel supply should be sufficient for at least 48 hours. Back-up sources for diesel fuel should be identified. The diesel plant repairman's name and telephone number are posted in the generator room.

2. If there is a "hurricane warning," hurricane panels should be installed in the "main laboratory buildings." All electrical devices and papers should be moved away from window areas to prevent water damage.

3. All noncritical electrical equipment should be disconnected. All "critical" equipment will be marked as such with a tag on the electric wire.

4. Any field equipment, tables, chairs, or other loose objects that might become flying projectiles during a storm should be secured or cleared from the ground.

5. All government vehicles should have full fuel tanks and should be securely parked in areas where trees will not fall on them and flooding will not occur.

6. The Director of SJL will communicate any last minute instructions to all employees.

7. If the employees are at the SJL, and a "hurricane warning" is issued, the Director may dismiss employees once the above precautionary actions have been taken.

8. If a "hurricane warning" is issued on regular work days before employees report to work, all employees should contact SJL for instructions or listen to WOSO (1030 AM) radio station.

After the disaster event has passed:

1. The Director, Section Chiefs, Biosafety Officer, and the Program Analyst will return to the laboratory as soon as possible.

2. Care should be taken that there are no fallen electric cables or wires. If they are found, call the electric company for assistance (Tel 721-1212) or the Civil Defense (724-0124).

3. If a fire occurs (as a result of lighting, etc.), the fire department should be contacted. Firemen should be advised as to the location of flammable materials and infectious materials (Revco Freezers) with proper instructions.

4. Ensure that both power generators are working with at least 1/4 of a tank (sufficient fuel for at least 24 hours).

5. Ensure that all freezers, refrigerators, and incubators connected to the emergency power are working.

6. Inspect the laboratory building and grounds for damage assessment and post warning signs when necessary.

7. If there is telephone service available, establish communications with SJL employees, CDC (Ft. Collins and Atlanta), municipal, state, and other federal offices.

**If an employee is alone, or working on a weekend, holiday, or after normal duty hours and a disaster occurs he/she should:**

1. Attempt to communicate with the Director, Biosafety Officer, Program Analyst, and Section Chiefs.

2. In case of a fire, call the fire department (Tel 343-2330) and follow the fire plan (see attachment).

3. If the employee is injured, but able to communicate, the person must seek immediate medical attention by contacting any other employee on the premises or calling out for assistance.

After communication with other offices:

1. When requested, provide technical assistance to federal, state, and municipal officials in the areas of communicable and vector-borne disease control and handling of related environmental health hazards.

2. When requested, assist in establishing a public health surveillance system.

# APPENDIX A

## Biological Safety Cabinets

Biological safety cabinets are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. Each of the three types -- Class I, II, III -- has performance characteristics, which are described in this appendix. In addition to the design, construction, and performance standards for vertical laminar flow biological safety cabinets (Class II), the National Sanitation Foundation has also developed a list of such products that meet the reference standard. Utilization of this standard and list should be the first step in selecting a biological safety cabinet.

Class I and II biological safety cabinets, when used in conjunction with good microbiological techniques, provide an effective partial containment system for safe manipulation of moderate and high-risk microorganisms (i.e., Biosafety Level 2 and 3 agents). Both Class I and II biological cabinets have comparable inward face velocities (75 linear feet per minute) and provide comparable levels of containment in protecting the laboratory worker and the immediate laboratory environment from infectious aerosols generated within the cabinet.

It is imperative that Class I and II biological safety cabinets are tested and certified *in situ* at the time of installation within the laboratory, at any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model, but does not supersede the critical certification prior to use in the laboratory.

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the biological safety cabinets. The slide-sound training film developed by NIH (Effective Use of the Laminar Flow Biological Safety Cabinet) provides a thorough training and orientation guide. Of particular note are those activities that may disrupt the inward directional airflow through the work opening of Class I and II cabinets. Repeated insertion and withdrawal of the workers' arms in and from the work chamber, or brisk walking past the BSC while it is in use cause aerosolized particles to escape from within the cabinet. Strict adherence to recommended practices for the use of biological safety cabinets is as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

Horizontal laminar flow "clean benches" are present in a number of clinical, pharmacy, and laboratory facilities. These "clean benches" provide a high-quality environment within the work chamber for manipulating nonhazardous materials. Caution: Since the operator sits in the immediate downstream of exhaust from the "clean bench," this equipment must never be used for the handling of toxic, infectious, or sensitizing materials.

The Class I biological safety cabinet is an open-front, negative-pressure, ventilated cabinet with a minimum inward face velocity at the work opening of at least 75 feet per minute. The exhaust air from the cabinet is also filtered by HEPA filters. Design, construction, and performance standards for Class II cabinets have been developed by and are available from the National Sanitation Foundation, Ann Arbor, Michigan.

Personnel protection provided by Class I and Class II cabinets is dependent on the inward airflow. Since the face velocities are similar, they generally provide an equivalent level of personnel protection. The use of these cabinets alone, however, is not appropriate for

containment of highest risk infectious agents because aerosols may accidentally escape through the open front.

The use of a Class II cabinet in the microbiological laboratory offers the additional capability and advantage of protecting materials contained within it from extraneous airborne contaminants. This capability is provided by the HEPA-filtered, recirculated, mass airflow within the work space.

The primary biohazard arising from sonication of infectious material (mosquitoes, cell culture, tissues, etc.) is the creation of aerosols. Proper containment and protective clothing must be used at all times. Sonication of infectious material should always be carried out within a vertical-flow laminar-flow biosafety cabinet, Class II or higher. Good safety cabinet practices should be employed. Protective laboratory coats and gloves should also be worn.

**Ultracentrifuge Safety**

Two biohazards can result from improper use of the ultracentrifuge: 1) infectious aerosols can be produced during processing in the ultracentrifuge, and 2) rotor or tube failure can lead not only to infectious aerosols but also to physical danger.

As with high speed centrifugation, aerosols can be produced during opening of rotors or buckets. The rotor is a sealed vessel during centrifugation, but it should be loaded and opened in a vertical-laminar flow safety cabinet if used with infectious material. Gloves and laboratory coats should be used at all times. Because it is impossible to detect sample leakage during centrifugation until the rotor chamber is opened, it is important to also wear eye protection and a surgical mask when retrieving a rotor upon completion of the centrifugation. Because the ultracentrifuge is useful for purification and concentration of virus suspensions, extra care should be taken when working with concentrated virus preparations. Remember that a tissue culture seed with 7 to 8 logs of virus can yield a purified virus suspension of 9 to 10 logs. This is a highly infectious inoculum and should be treated with due respect. All handling of concentrated virus suspensions or gradients containing such must be performed in a laminar flow hood. Special care should be taken when removing concentrated virus bands from density gradients. Eye protection should be worn during this stage to prevent accidental eye splashes.

The main causes of ultracentrifuge failure are improperly balanced centrifuge tubes, sample leakage which results in imbalance, and rotor fatigue.

Balancing Tubes

It is imperative that all tubes be balanced against another tube of fluid on a pan balance. Adjusting fluid levels by eye will not work with the ultracentrifuge! These balanced tubes must be placed opposite each other in the rotor. Remember that a swinging bucket rotor has well-defined bucket placement holes that are numbered. The hole number matches the bucket number. Do not place buckets in a wrong rotor hole! Do not run the rotor with fewer than three buckets. If you have only two filled buckets, use also two more empty buckets, placing them opposite each other in their appropriate rotor holes.

Sample Leakage

When filling tubes, do not overfill or underfill. Both conditions could lead to sample leakage or tube collapse. Fill tubes within 1/4 inch of the top. When all tubes have been properly

balanced and loaded into the rotor, centrifugation can commence. Watch the ultracentrifuge until it has reached 10,000 rpm. Balance problems will usually occur before 10,000 rpm. If balance problem are detected, shut down the centrifugation and readjust balance. Be very careful. Use appropriate protective gear, as outlined above, during sample removal.

Rotor Failure

Rotor failure can occur for a number of reasons. The primary causes of rotor failure are unbalanced tubes (see above), rotor overspeed, or rotor fatigue. Rotor failure is a serious and dangerous problem. All modern rotors have their own code. Be sure to run rotors only in centrifuges rated with the same code. It is imperative to observe these codes! When a rotor fails and breaks apart or comes off the ultracentrifuge spindle, the can is designed to contain all rotor pieces. If a rotor fails in a machine that is not rated for it, there is a good possibility that penetration will occur, and deadly flying shrapnel might result! To avoid rotor failure due to overspeed, remember to check rotors for the presence of an overspeed decal on the rotor bottom. The overspeed decal helps the instrument detect the proper speed settings for each rotor. Do not attempt to run rotors faster than their designed speeds (e.g., SW41 rotor is designed for 41,000 rpm maximum speed). Rotors may also be designed with "knock-out" pins. These pins are found towards the rotor bottom. If a rotor goes overspeed, these pins are designed to release. The ultracentrifuge detects the pin release and shuts down. Rotors are routinely derated. This means that as a rotor ages (determined by usage) the maximum speed is lowered. If you are unsure of an older rotor's derated maximum speed, contact the centrifuge supervisor. If rotor failure occurs, shut-off the machine and leave the room immediately! Get your supervisor! When opening a machine that has failed, be prepared for an infectious aerosol! Remember to let the machine and pieces cool before touching them because the friction caused in a rotor failure generates considerable heat. Decontaminate the rotor pieces and ultracentrifuge thoroughly. Save the rotor pieces and call your supervisor.

**Operating Procedures for Castle Steam Autoclaves**

Loading Sterilizer

Place a 1/2-1 inch piece of autoclave tape on the lids or covers of stainless steel pans containing infectious equipment and materials. This tape has clear stripes before sterilization, which turn black following sterilization. Carefully stack pans inside of sterilizers so they do not fall over. This would result in contamination of the immediate area. Caution must be taken so that liquids in pans with lids, white pans, and glassware are not spilled outside the autoclave when loading. Do not overload the autoclave. Contents of an overloaded autoclave fall out and scald the operator when the door is opened. Before adding a dirty container to the autoclave, determine whether other contents are also dirty. If not, you will contaminate a sterile load.

Operating Sterilizer

When sterilizing containers with liquids, press the liquid button; press dry goods button when sterilizing pans or equipment without liquids. When the operating needle moves to the load position, place materials to be sterilized into the autoclave. Close the door, turn the lock handle clockwise (located on hub of door closure mechanism), and turn long gray handles clockwise, until you cannot turn them anymore. The door must be sealed tight or steam will escape from the chamber. For most sterilization procedures, set the timer at 30-45 minutes. Sterilization cycle will not commence until jacket pressure and chamber pressure are at 20 pounds. The temperature should read 250˚ in chamber. The timing light comes on and the

sterilizing timer moves counterclockwise towards zero. When it reaches zero, the sterilization cycle is complete. The timing light goes out and the cooling light comes on. The chamber pressure gradually drops to zero and the pointer on the operating handle moves to off. The cooling light goes out, the sterile light comes on, and a buzzer sounds. Press the off button to silence buzzer. A manual control handle is used in manual operation of the sterilizer. The dial of this control is labeled off, load, sterilize, liquid cool, and dry/vent. The manual control handle pointer and the signal lights indicate what phase the unit is in during the sterilizing cycle.

Unloading Sterilizer

Use insulated gloves to remove material from the sterilizer. Check autoclave tape on vessels before removing. **DO NOT** open or remove any containers where the autoclave tape stripes have not changed to black. If the tapes have not changed, put on fresh tape, and resterilize the pan(s). Make certain that the autoclave has not malfunctioned.

## Protocol for Infected Rabbit Feedings

1. 5-6 lb female rabbits will be anesthetized with 0.5ml/5lb rabbit of acepromazine maleate and counted numbers of ticks (maximum 25 males and 25 females per animal) will be placed into ear bags and taped to the animal. All feedings will take place in Building 4.

2. Ticks will be allowed to feed on rabbits for a maximum of 10 days. Ear bags will be checked for replete ticks starting on Day 6. Ear bags will be opened and checked a minimum number of times. All ticks placed on the rabbit will be accounted for by counting onto and off the rabbit, and a log kept.

3. Upon the termination of feedings, the entire rabbit will be treated with a commercial insecticide (Ferti-lome home and pet spray) containing 0.02% pyrethrin. The cage and bedding will be similarly treated.

4. 36-48 hrs after treatment, the cage and rabbit will be brought back to the main animal facility. The cage and bedding will be autoclaved immediately. The rabbit will be placed in a standard rabbit cage. Pyrethrins have a residual activity of <24 hrs.

## Hantavirus Biosafety with Human Specimens and Wild Rodents

Introduction

Laboratory manipulations of live wild rodents, their carcasses, tissues, blood and/or sera are sometimes performed especially by the plague and Lyme groups of the Bacterial Zoonoses and Medical Entomology and Ecology Branches. During the summer of 1993, *Peromyscus sp.* (wild mice such as the deer mouse) were implicated in the transmission of hantaviruses to humans in the western U.S. Seroprevalence studies of hantavirus antibodies in southwestern deer mice have shown that 23% of these rodents have been/are infected. These strains of hantavirus have been associated with a severe, often fatal
pneumonia in adult humans. Asian and European hantaviruses produce a febrile hemorrhagic

disease which primarily involves the kidneys and liver.

In both forms of disease, transmission to humans is thought to occur from hantavirus contaminated saliva, feces and urine of infected rodents which, although actively shedding virus, do not appear to be ill from the infection. Aerosol transmission of the Asian hantaviruses appears to occur readily around caged infected rodents, and aerosolized fecal, urinary, or salivary material are probably important in transmission of the U.S. strains as well. The presence of virus in the saliva suggests that transmission by rodent bites can occur from rodent to rodent or rodent to human. Bites of rodents infected with the Asian hantaviruses are known to infect humans. Arthropod vectors are not known to have a role in the transmission of hantaviruses.

Clinical specimens (i.e. tracheal secretions, serum, blood, and tissues) from potential hantavirus infected patients may also represent a biohazard to laboratorians. Such specimens must be handled in a containment environment or, if possible, subjected to virus inactivation procedures that will render them safe to manipulate without containment.

Hantaviruses have lipid envelopes that are susceptible to most disinfectants (e.g. dilute sodium hypochlorite solutions, 70% ethanol, or most general-purpose household disinfectants containing lipid solvents as an ingredient).

**Human Specimen Precautions**

Human specimens which should arouse suspicion as potentially infected with hantavirus will be obtained from cases of hemorrhagic disease or pneumonia (and at DVBID from suspected cases of plague pneumonia), especially cases described as adult acute respiratory distress syndrome. Sputum, blood, serum, and/or tissues from such cases should either be inactivated by heat or gamma irradiation for manipulations in a BSL 2 facility or handled in a laminar flow biosafety cabinet LFBSC in a BSL 3 facility. Care should be taken to avoid production of aerosols from nonirradiated material. Packaging which contains potential hantavirus infected materials should only be opened in a LFBSC.

**Wild and Laboratory Rodent Precautions**

Until the rodent host range of hantavirus infections has been established with greater certainty, all wild rodents should be handled as potential sources of hantavirus infection. Similarly, laboratory rodents injected with or otherwise exposed to blood, blood components, or other tissues from wild rodents must be considered as potentially hantavirus infected. Either wild or laboratory animals infected with hantaviruses represent a clear risk of aerosol transmission from infected urine, feces and saliva on bedding material; accidental laboratory infections by this route are well established. Ectoparasites are not known to play any role in hantavirus transmission, so laboratory animals whose exposures are solely to ectoparasites (fleas, macerated fleas, or ticks) do not need to be treated as potentially infected with hantaviruses.

**Maintenance of Potential Hantavirus Infected Rodents**

Wild or laboratory rodents potentially infected with hantaviruses must be maintained in a designated area in specially designed air handling units. This will take the form of Biogard containment units or individual microisolator cages. The currently designated housing facility for these rodents will be Room 119D. The following biosafety steps should be rigorously maintained:

1. Access to this room will be controlled and limited to designated personnel. During use of 119D as a Hantavirus containment area, the door from the suite anteroom into 119D will remain locked and blocked. Entry into 119D when it is being used as a hantavirus containment laboratory will be through the newly constructed doorway between 119C and D. The door to the 119 suite anteroom from the hall will remain locked at all times when hantavirus work is in progress.

2. Individuals entering the hantavirus containment area must first don full face masks (PAPRS), jump suits, gloves, and foot/shoe covers. Check to see that the PAPRS power pack is functioning. An additional fully charged or charging powerpack should be in 119D at all times the facility is in use for hantavirus containment to serve as a backup.

3. Tissues, blood components, etc., obtained from post-mortem preparation of field collected rodent carcasses (including tissue grinding) which are to be prepared for inoculation into laboratory animals, will be done in the laminar flow hood in 119D.

4. Needle inoculations of live laboratory rodents with these preparations will be done using the single hand needle manipulation technique to minimize risk of accidental autoinoculation.

5. Materials such as needles, syringes, surgical instruments, mortars, pestles, glassware, water bottles, sippers, cages with bedding, and other contaminated articles will be chemically disinfected for at least 12 hours (see above for suitable disinfectants).

6. Chemically disinfected materials will be transfered from 119D in a closed transporter or sealed double biohazard bags to the autoclave immediately outside the door to the 119 suite of rooms. In the event that the autoclave outside 119 suite is in use, materials for autoclaving should be left in their double bags inside 119C.

7. Live animals should be handled with a clean heavy glove (never used in the manipulations of potentially infected animals) or other techniques to minimize the risk of infectious bites when they are being inoculated or moved to clean cages.

8. Because hantaviruses can be transferred from cage to cage by the contaminated hands and equipment of the individuals manipulating animals, gloves and other equipment should be changed between cages of potentially infected animals.

9. Clothing worn in the hantavirus containment facility should be removed using hand protection and bagged for autoclaving prior to departing the facility.

10. Room 119D will be decontaminated by appropriate methods under the supervision of the Division Biosafety Officer and used as a Biosafety Level 2 Animal facility when wild animal work is not in progress.

**PROCEDURES FOR HANDLING RODENTS IN HANTAVIRUS ENDEMIC AREAS OF THE UNITED STATES: RECOMMENDATIONS FOR DVBID PERSONNEL CONDUCTING COMBINED FIELD STUDIES FOR HANTAVIRUS, LYME DISEASE AND PLAGUE**

## Trapping

Set out Sherman and Tomahawk traps in appropriate numbers and configuration according to pre-established design or terrain characteristics. No special precautions for personal protective measures need be followed during this activity.

## Collecting Trapped Rodents

Before checking traps for rodent captures, each person is required to put on latex gloves and carry two medium to large plastic bags (one placed inside the other). Respirators are not necessary for this operation, but the fitted half-mask type may be worn if the worker(s) so desires. Each person handling traps while checking for rodent captures should carry along one or two extra pairs of gloves as the metal traps may snag and tear the latex.

Closed Sherman traps should be properly labelled with collection data and placed directly into the double plastic bag. Do not open the door of the trap to identify the animal or to determine whether the trap is merely sprung but empty. This action can potentially expose the field worker to aerosols caused by the animal scurrying around inside the trap. Handle animals collected in wire Tomahawk traps in the same manner.

After picking up the rodent captures, close off the plastic carrying bags containing closed traps by expressing the air from the bag in a direction away and downwind from the face and other personnel. Be aware that rodent mortality may be high if left unattended during hot weather and care should be taken to protect bagged animals from heat stress. Safely remove the latex gloves and place them in an appropriate decontamination bag for subsequent autoclaving or incineration.

## Transporting Trapped Rodents

If trapped animals must be transported for any distance to a central processing site, the bagged rodents are not to be placed inside the passenger compartment of vehicles. Personnel and captured rodents can be separated according to the type of field vehicle being used. If a pickup is utilized, placing the bagged animals in the bed (with or without a topper shell) during transport is adequate. If a sedan is used and the trunk is totally separate from the passenger compartment, transporting the bagged rodents in the trunk is acceptable. If vehicles without separate cargo compartments are used, bagged rodents should be secured outside the vehicle on a luggage rack during transport.

## Processing Captured Rodents

Upon arrival at the field processing site, put on a pair of latex gloves before removing bagged rodents from the vehicle. Place animals in a protected location.

Set up and arrange supplies, instruments and equipment which will be used to process animals for blood samples, ectoparasite collection, and ear biopsy specimens. Prepare three 5

gallon buckets for trap sterilization and rinsing. Each bucket should contain 4 gallons of water. One bucket will contain 5-10% Lysol and each trap from which an animal is removed will be immersed in this solution for 5-10 minutes. The other two buckets will be used successively to rinse the disinfected traps.

Prior to handling rodents, field staff will don a disposable surgical gown, surgical booties to cover footwear, double latex examination gloves and an OSHA approved respirator. Remove the animal from its trap and administer either ketamine chloride or methoxyflurane for anesthesia. The collection of specimens from the rodent should follow this sequence:

1. Remove ectoparasites;
2. Harvest ear tissue and place in disinfectant; and
3. Collect blood sample from postorbital capillary sinus with heparinized capillary tube.

Direct heart puncture with needle and syringe may be necessary for larger animals, but special care must be used because of potential sticks with sharps.

Fleas can be stored in vials of 70% alcohol; ticks should be kept alive in standard moistened tick vials; disinfected ear tissue biopsy specimens will be placed in BSK-II culture media and stored at ambient temperature; whole blood samples will be frozen in dry ice or liquid nitrogen.

Additional precautions to be aware of while processing rodents include:

1. If an outer latex glove becomes cut or torn replace it immediately;
2. Clean all instruments with liquid disinfectant and flame from alcohol burner;
3. Soak working surface areas contaminated with urine, feces, and/or blood with 5-10% solutions of Lysol or sodium hypochlorite; and
4. Disinfect latex gloves in Lysol or sodium hypochlorite if they become contaminated with rodent excreta or blood during specimen collection.

**Disposal of Contaminated Materials**

All paper, plastic bags, packaging, rubber gloves, surgical gowns and booties, etc. will be placed in doubled biohazard bags to be incinerated. Sharps and capillary tubes will be placed in standard autoclavable sharps containers. Incineration and autoclaving will be done at DVBID in Fort Collins or at prearranged locations if working at remote sites.

Since organ tissues will not be taken for virus isolation, rodents can be returned to the area of capture. Animals which may expire during processing can be buried at the processing site if it is in a rural area, or placed in a double biohazard bag and frozen for return to DVBID if in an urban area.

**Specimen Processing in the Laboratory**

Risk of exposure to hantavirus in the DVBID laboratories during routine testing of ectoparasites is extremely low. P2 facilities will be adequate for these activities.

Hantavirus does not replicate in BSK-H culture media. Therefore, cultures containing ear tissue samples can be processed using standard procedures for incubating and examining cultures for spirochetes with personal safety precautions of laminar flow hood, gloves and lab

coat.

Inoculation of white lab mice with cultured original isolate spirochetes to determine infectivity will pose no change of procedure for animal handling and care from that now in effect.